

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
26 April 2007 (26.04.2007)

PCT

(10) International Publication Number
WO 2007/045243 A2

(51) International Patent Classification:
C07K 14/43 (2006.01)

(21) International Application Number:
PCT/DK2006/000580

(22) International Filing Date: 16 October 2006 (16.10.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PA 2005 01455 17 October 2005 (17.10.2005) DK

(71) Applicant (for all designated States except US): **ENKAM PHARMACEUTICALS A/S** [DK/DK]; Fruebjergvej 3, DK-2100 Copenhagen Ø (DK).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **BEREZIN, Vladimir** [UA/DK]; Nørrebrogade 223, 1.th., DK-2200 Copenhagen N (DK). **BOCK, Elisabeth** [DK/DK]; Tonysvej 20, DK-2920 Charlottenlund (DK).

(74) Agent: **HØLBERG A/S**, St. Kongensgade 59A, DK-1264 Copenhagen K (DK).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: NOVEL FGF RECEPTOR BINDING COMPOUNDS COMPRISING PEPTIDE FRAGMENTS OF NEURAL CELL ADHESION MOLECULE L1

(57) Abstract: The present invention relates to novel peptide compounds capable of interacting with Fibroblast Growth Factor Receptor (FGFR) comprising an amino acid sequence of 5 to 105 amino acid residues which comprises an amino acid motif of the formula $x^1-(x)_n-x^p-(x)_n-x^1$, wherein x^1 is a basic amino acid residue, x^p is a hydrophobic amino acid residue and $(x)_n$ is a sequence of any amino acid residues, wherein n is an integer from 0 to 3. Preferably, the compounds of the invention comprise a fragment of neural cell adhesion molecule L1. The invention also relates to uses of the compounds for treatment and/or prevention of different pathological conditions wherein FGFR and/or L1 play a role in pathology and/or recovery from the disease. Accordingly, pharmaceutical compositions comprising the compounds of the invention are also concerned.-



WO 2007/045243 A2

Novel FGF receptor binding compounds comprising peptide fragments of neural cell adhesion molecule L1

Field of invention

5

The present invention relates to novel peptide compounds capable of binding to fibroblast growth factor receptor (FGFR) and activating said receptor. Novel compounds of the invention comprise peptide fragments comprising FGFR binding motif $x^-(x)_n-x^p-(x)_n-x^-$, wherein x^- is a basic amino acid residue, x^p is a hydrophobic amino acid residue and $(x)_n$ is a sequence of any amino acid residues wherein n is a integer from 0 to 3. Preferably, a compound of the invention comprises a fragment of neural cell adhesion molecule L1 which is capable of binding to FGFR and activating the receptor. The invention discloses the amino acid sequences of the FGFR binding fragments of L1 and features pharmaceutical compositions comprising thereof. Invention also relates to uses of the compounds and pharmaceutical compositions for treatment of different pathological conditions, wherein FGFR and/or L1 play a role in pathology and/or recovery from the disease. Antibodies which are capable of binding to epitopes comprising peptide sequences of the invention are also concerned.

20

Background of invention

25

30

35

Brain plasticity and the mechanisms controlling plasticity are central to learning and memory as well as the recovery of function after brain injury. While it is clear that neurotrophic factors are one of the molecular classes that continue to regulate brain plasticity in the adult central nervous system (CNS), less appreciated but equally profound is the role of cell adhesion molecules (CAMs) in plasticity mechanisms such as long term potentiation, preservation of neurons and regeneration. Ironically, however, CAMs can also reorganise the extra-cellular space and cause disturbances that drive the development of brain pathology in conditions such as Alzheimer's disease and multiple sclerosis. Candidate molecules include the amyloid precursor protein, which shares many properties of a classical CAM and beta-amyloid, which can masquerade as a pseudo CAM. Beta-Amyloid serves as a nidus for the formation of senile plaques in Alzheimer's disease and like CAMs provides an environment for organising neurotrophic factors and other CAMs. Inflammatory responses evolve in this environment and can initiate a vicious cycle of perpetuated

neuronal damage that is mediated by microglia, complement and other factors (Cotman et al. (1998) *Prog Neurobiol.* 55:659-69).

Neural cell adhesion molecules (CAMs) of the immunoglobulin superfamily nucleate and maintain groups of cells at key sites during early development and in the adult. In addition to their adhesive properties, CAMs homophilic and heterophilic interactions can affect intracellular signalling. Their ability to influence developmental events, including cell migration, proliferation, and differentiation may therefore result from both their adhesive and signalling properties.

Neural cell adhesion molecule L1 plays a pivotal role during development of the nervous system, mediating binding between neuronal cells and stimulating axonal outgrowth and fasciculation. L1 consists of six immunoglobulin (Ig) modules in the amino-terminal region, followed by five fibronectin type III (F3) modules, a transmembrane domain, and a highly conserved cytoplasmic tail (Nybroe and Bock, 1990). L1 is known to mediate cell-cell interactions by a homophilic binding, i.e. L1 on one cell binds to L1 on an adjacent cell (Grumet and Edelman, 1984; Lemmon V et al., 1989, Doherty et al., 1995). It is also capable of heterophilic binding to various cell surface molecules including the neural cell adhesion molecule (NCAM) (Horstkorte et al., 1993), the axon-associated cell adhesion molecule TAG-1/axonin-1 (Kuhn et al., 1991; Felsenfeld et al., 1994), the glycosyl-phosphatidylinositol-anchored molecule HAS/CD24 (Sammar et al., 1997) and the nervous tissue-specific chondroitin sulphate proteoglycans neurocan and phosphacan (Margolis et al., 1996), and it also binds to components of the extracellular matrix.

It has been shown that neurite outgrowth stimulated by L1 involves activating of intracellular signaling cascades dependent on tyrosine kinase activity of fibroblast growth factor receptor (FGFR) (Williams et al., 1994). Based on an indirect evidence, it has been suggested that L1 can activate FGFR by interacting with the receptor extracellularly, however, neither evidence of the interaction has been demonstrated, nor binding site(s) of the interacting molecules have been identified.

Fibroblast growth factor receptors (FGFRs) are a family of at least four closely related receptor protein tyrosine kinases, FGFR 1, FGFR 2, FGFR 3 and FGFR 4, consisting extracellularly of three Ig-like modules and intracellularly of a split tyro-

sine-kinase module (Powers et al. (2000) *Endocr Relat Cancer* 7:165-97). The receptors are known to be key regulators of morphogenesis, development, angiogenesis, and wound healing. FGFR activation and signalling are dependent on dimerization of the receptor which is induced by high affinity binding of FGFR natural ligand, fibroblast growth factor (FGF), and it also requires participation of cell surface heparin or heparan sulphate proteoglycans. Fibroblast growth factors (FGFs) and their receptors constitute an elaborate signaling system that participates in many developmental and repair processes of virtually all mammalian tissues, in particular, they play a prominent role in functioning of the peripheral and central neural system. Thus, among 23 members of the FGF family, ten have been identified in the brain (Jungnickel et al, (2004) *Mol Cell Neurosci*. 25:21-9; Reuss and von Bohlen und Halbach (2003) *Cell Tissue Res*. 313:139-57).

A large body of evidence accumulated by now indicates that FGFR receptor is involved in intracellular signal transduction associated with neural cell adhesion molecules NCAM, L1 and N-cadherin (Williams et al. (1994) *Neuron* 13:83-94). NCAM has recently been regarded as a member of a new class of putative alternative ligands of FGFR, low affinity ligands (Kiselyov et al. (2005) *J Neurochem* 94:1169-1179). There has been obtained evidence for a direct interaction between NCAM and the receptor (Kiselyov et al. (2003) *Structure (Camb)* 11:691-701). The identified NCAM fragment having the sequence EVYVVAENQQGKSKA (FGL peptide) involved in the direct interaction between NCAM and FGFR has been suggested as a new candidate drug for the treatment of a variety of pathologic conditions where the activity of FGFR may play a key role (WO 03/016351). WO 03/016351 describes some biological effects of the FGL peptide due to binding and activating FGFR.

L1 has been broadly studied as a neurite outgrowth stimulating and cell survival promoting cell adhesion molecule (Haspel et al. (2000) *J Neurobiol* 15:287-302; Roonprapurt et al. (2003) *J Neurotrauma* 20:871-882; Wiencken-Barger et al. *Cereb Cortex* (2004) 14:121-131; Loers et al. (2005) *J Neurochem* 92:1463-1476). It has also been regarded important for neural cell precursor proliferation, differentiation and transmitter phenotype subtype generation (Dihne et al. (2003) *J Neurosci* 23:6638-6650), and it is known to play a role in synaptic plasticity (Saghatelyan et al. (2004) *Mol Cell Neurosci* 26:191-203).

A number of studies have been performed to study the involvement of different regions of L1 molecule in execution of multiple biological activities by L1 and to associate the cellular responses mediated by L1 with particular intracellular signal transduction pathways. Thus, it has been demonstrated that the Ig modules 1-4 of L1 are critical for homophilic binding and neurite outgrowth (Haspel et al (2000) *J Neurobiol* 42:287-302). An antibody directed to an epitope between F3 domains 2 and 3 has been shown to promote signal transduction and concomitant neurite extension (Appel et al (1995) *J. Neurobiol.* 28:297-312). Holm et al. (1995) demonstrated that certain FN-like domain fragments have a capacity for homoaggregation, suggesting that one or more of the FN-like domains may have the potential for self-association, perhaps leading to the clustering of L1 at the cell surface; such clustering may in turn be subject to conformational constraints imposed by the globular configuration of the FN-like repeats (*J. Neurosci. Res* 42:9-20). The whole ectodomain of L1 both as a soluble molecule, and substrate is important for the promotion of neural cell survival and stimulation of neurite outgrowth (Loers et al. (2005) *J Neurochem* 92:1463-1476; Naus et al. (2004) *J Biol Chem* 279:16083-90).

Soluble molecules of L1 have been suggested for use as neurite outgrowth and survival promoting compounds in therapeutic applications (US 6,576,607). However, the usage of such molecules for medical applications is limited as the L1 ectodomain polypeptide is very long (constituting 1100 amino acids (Swissprot P32004)).

Identification of functionally important regions of L1 polypeptide and production of these regions as individual peptide fragments which are capable of mimicking the biological function of the whole L1 ectodomain polypeptide, seems might solve the problems arising when a medical application of L1 is concerned. A beneficial input may also give the identification of binding sites in L1 which are involved in interaction with different L1 binding molecules. It would advantageously provide new effective drugs for the treatment of diseases where the molecules interacting with L1 are concerned, e. g. FGFR receptor. However, information about structural requirements for the functional capabilities of L1 molecule is insufficiently disclosed in the prior art, if available at all.

Summary of invention

The present invention relates to identifying FGFR binding sites in L1 and providing compounds comprising peptide fragments derived from these binding sites. Novel compounds of the invention are FGFR binding compounds which are capable of modulating FGFR activity.

5

Thus, it is a first objection of the invention to provide novel compounds comprising peptide sequences capable of binding to FGFR and modulating the FGFR activity.

Compounds of the invention comprise peptide sequences which comprise FGFR binding motif $x^-(x)_n-x^p-(x)_n-x^-$, wherein x^- is a basic amino acid residue, x^p is a hydrophobic amino acid residue and $(x)_n$ is a sequence of any amino acid residues wherein n is a integer from 0 to 3. Preferably compounds of the invention are at most 105 amino acids long and comprise a fragment of neural cell adhesion molecule L1 which is capable of binding FGFR and activating the receptor. The invention discloses amino acid sequences of the FGFR binding fragments of L1 and features pharmaceutical compositions comprising thereof. Invention also relates to uses of the compounds and pharmaceutical compositions comprising thereof for the treatment of different pathological conditions, wherein FGFR and/or L1 play a role in pathology and/or recovery from the disease, for example for

10

15

20

25

30

35

- a) treatment of conditions of the central and peripheral nervous system associated with postoperative nerve damage, traumatic nerve damage, impaired myelination of nerve fibers, postischaemic damage, e.g. resulting from a stroke, Parkinson's disease, Alzheimer's disease, Huntington's disease, dementias such as multiinfarct dementia, sclerosis, nerve degeneration associated with diabetes mellitus, disorders affecting the circadian clock or neuro-muscular transmission, and schizophrenia, mood disorders, such as manic depression;
- b) treatment of diseases or conditions of the muscles including conditions with impaired function of neuro-muscular connections, such as after organ transplantation, or such as genetic or traumatic atrophic muscle disorders; or for treatment of diseases or conditions of various organs, such as degenerative conditions of the gonads, of the pancreas such as diabetes mellitus type I and II, of the kidney such as nephrosis and of the heart, liver and bowel;
- c) promotion of wound-healing;
- d) prevention of death of heart muscle cells, such as after acute myocardial infarction;

- e) promotion of revascularisation;
- f) stimulation of the ability to learn and/or the short and/or long-term memory;
- g) prevention of cell death due to ischemia;
- h) prevention of body damages due to alcohol consumption;
- 5 i) treatment of prion diseases;
- j) treatment of cancer.

Antibodies, which are capable of binding to epitopes comprising peptide sequences of the invention, are also concerned by the invention.

10

Description of Drawings

Fig. 1. Binding of L1 F3 modules I-V to FGFR1 Ig modules II-III. The binding was studied by means of SPR analysis. Approx. 3000 resonance units (RU) of the FGFR1 modules were immobilized on the sensor chip. Combined L1 F3 modules I-V or combined NCAM Ig modules I-II (ctrl) were injected into the sensor chip at the specified concentrations. The binding is given as a response difference between the binding to the sensor chip with the immobilized FGFR1 modules and a blank sensor chip (unspecific binding). Only a very low unspecific binding was detected for the above mentioned proteins at all concentrations used in this study. Four-five independent experiments were performed using different preparations of all proteins.

Fig. 2. Demonstration of an ATP-induced increase of binding between FGFR1 Ig modules II-III and L1 F3 modules I-V. The binding was studied by SPR analysis. Approx. 3000 resonance units (RU) of the FGFR1 modules were immobilized on the sensor chip. Three independent experiments for each concentration of ATP (AMP-PCP, AMP or GTP) were performed. A, B, C, D) Binding of 14 μ M F3 modules I-V to the FGFR1 modules in the presence of ATP (A), AMP-PCP (B), AMP (C) and GTP (D) at different concentrations.

Fig. 3. Demonstration of an effect of ATP, AMP-PCP, GTP, AMP on the binding between FGFR1 Ig modules II-III and L1 F3 modules I-V. A) The equilibrium dissociation constant K_D of the interaction between the F3 modules and FGFR1 before (Ctr) and after the addition of ATP, AMP-PCP, GTP or AMP. B) A plot of the initial binding rate V_0 of binding of the F3 modules to FGFR1 versus the ATP

concentration was used to calculate the equilibrium dissociation constant K_D of the interaction between the F3 modules and ATP.

Fig. 4. Effect of the L1 F3 modules I-V on phosphorylation of FGFR1 and immunoprecipitation of L1 by FGFR1.

5 A) TREX/FLP-IN cells stably transfected with FGFR1 containing a C-terminal StrepII-tag were stimulated for 15 min with nothing (lane 1), 4 μ M (lane 2), 10 μ M (lane 3) and 30 μ M (lane 4) L1 F3 modules I-V. After stimulation, FGFR1 was immunopurified using anti-phosphotyrosine antibodies and then analyzed by immunoblotting using antibodies against the StrepII-tag. B) TREX/FLP-IN cells stably transfected with FGFR1 containing a C-
10 terminal StrepII-tag were stimulated for 15 min with 25 μ M NCAM Ig modules I-II (as a control, lane 1) and 10 μ M L1 F3 modules I-V (lane 2). A total of 9 experiments have been done. All experiments show phosphorylation of FGFR1 upon the treatment with the L1 F3 modules I-V.

15 **Fig. 5. Effect of L1 F3 modules I-V on neurite outgrowth from cerebellar neurons.** A) Neurite length versus the concentration of F3 modules I-V. B) Effect of an inhibitor of FGFR1, SU5402, on neurite outgrowth induced by 4 μ M F3 modules I-V. C, D) Neurons stimulated with 4 μ M F3 modules I-V in the presence of various concentrations of ATP (C) or AMP-PCP (D). E, F) Neurons stimulated with 1 μ M F3
20 modules I-V in the presence of various concentrations of ATP (E) or AMP-PCP (F). Four independent experiments were performed. Error bar represents one standard error of the mean. * and ** stand for statistical significance of $p < 0.05$ and $p < 0.01$, respectively, compared to Ctr. +, ++ and +++ stand for statistical significance of $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively, compared to the highest response.

25 **Fig. 6. Sequence of peptides representing loop regions of L1 F3 domains I-V.** 30 peptides representing all loops of the F3 I-V modules were synthesized and used to map binding sites between L1 and FGFR1. Shown are the loop areas of F3 I-V modules with parts of the adjacent beta strands.

30 **Fig. 7. Demonstration of binding between the FGFR1 and peptides derived from L1 F3 modules I-V.** The binding was studied by SPR analysis. Approx. 3000 resonance units (RU) of the FGFR1 modules were immobilized on the sensor chip. Three independent experiments were performed. A) Peptides bound to FGFR1
35 modules II-III (F1CDL- (8 μ M), F2BCL- (8 μ M), F3ABL- (40 μ M), F3CDL- (2 μ M),

F5BCL- (80 μ M), and F5FGL- (80 μ M) peptides). B) peptides bound to FGFR1 module II (F1CDL- (16 μ M), F2BCL- (16 μ M), F3CDL- (8 μ M), and F5BCL- (130 μ M) peptides).

5 **Fig. 8. Effect of the peptides bound to FGFR1 on phosphorylation of FGFR1.** Phosphorylation level versus concentration of the peptides bound to FGFR1. Four independent experiments were performed. Error bar represents one standard error of the mean. *, ** and *** stand for statistical significance of $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively, compared to PBS. +, ++ and +++ stand for statistical significance of $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively, compared to negative control (F1BCL-peptide that did not bind to FGFR1 and did not show dose-dependent phosphorylation of the receptor peptide).

15 **Fig. 9. Effect of the peptides bound to FGFR1 on neurite outgrowth from cerebellar neurons.** Neurite length versus the concentration of the peptides which bound to FGFR1. Four independent experiments were performed. Error bar represents one standard error of the mean. * and ** stand for statistical significance of $p < 0.05$ and $p < 0.01$, respectively, compared to Ctr (PBS).

20 **Fig. 10. A model of binding between L1 and FGFR1.** Possible compact conformation of L1 F3 modules was proposed where marked regions correspond to peptides, which bound to FGFR1, stimulated neurite outgrowth and phosphorylation of the receptor. Conformation of the dimer of FGFR1 is taken from the crystal structure of FGFR1 (Pellegrini *et al.*, 2000).

25 **Fig. 11. Gel-filtration experiments demonstrating possibility of a compact conformation of L1 F3 domains I-V.** A) Gel-filtration of L1 F3 domains I-V in PBS (blue color) and in PBS plus 1M NaCl (red color) using Superdex 200 pg gel-filtration column. B) Gel-filtration of BSA in PBS (blue color) and in PBS plus 1M NaCl (red color).

Detailed description of the invention

1. Compound

35 1.1. Peptide sequence

In the present context the standard one-letter code for amino acid residues as well as the standard three-letter code are applied. Abbreviations for amino acids are in accordance with the recommendations in the IUPAC-IUB Joint Commission on Biochemical Nomenclature Eur. J. Biochem, 1984, vol. 184, pp 9-37. Throughout the description and claims either the three letter code or the one letter code for natural amino acids are used. Where the L or D form has not been specified it is to be understood that the amino acid in question has the natural L form, cf. Pure & Appl. Chem. Vol. (56(5) pp 595-624 (1984) or the D form, so that the peptides formed may be constituted of amino acids of L form, D form, or a sequence of mixed L forms and D forms.

Where nothing is specified it is to be understood that the C-terminal amino acid of a peptide of the invention exists as the free carboxylic acid, this may also be specified as "-OH". However, the C-terminal amino acid of a compound of the invention may be the amidated derivative, which is indicated as "-NH₂". Where nothing else is stated the N-terminal amino acid of a polypeptide comprise a free amino-group, this may also be specified as "H-".

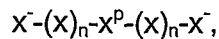
Where nothing else is specified amino acid can be selected from any amino acid, whether naturally occurring or not, such as alfa amino acids, beta amino acids, and/or gamma amino acids. Accordingly, the group comprises but are not limited to: A, V, L, I, P, F, W, M, G, S, T, C, Y, N, Q, D, E, K, R, H Aib, Nal, Sar, Orn, Lysine analogues, DAP, DAPA and 4Hyp.

Also, according to the invention modifications of the compounds/peptides may be performed, such as for example glycosylation and/or acetylation of the amino acids.

Basic amino acid residues are according to invention represented by the residues of amino acids H, K and R; acidic amino acid residues – by the residues of amino acids E and D; hydrophobic amino acid residues by the residues of amino acids A, L, I, V, M, F, Y and W; neutral, weakly hydrophobic - by P, A and G; neutral hydrophilic – by amino acid residues Q, N, S and T; cross-link forming by amino acid residue C.

In a first aspect the present invention relates to a compound capable of interacting with Fibroblast Growth Factor Receptor (FGFR) comprising an amino acid sequence

which comprises an amino acid motif of the formula



wherein

x^- is a basic amino acid residue,

5 x^p is a hydrophobic amino acid residue and

$(x)_n$ is a sequence of any amino acid residues, wherein n is an integer from 0 to 3.

According to the invention x^- may be any basic amino acid residue independently selected from K, R or H, x^p may be any hydrophobic amino acid residue independently selected from A, V, L, I, P, F, W, M or Y, and $(x)_n$ is a sequence of any amino acid residues, wherein n is an integer from 0 to 3. However, preferred motifs are, those wherein the residues x^- and x^p and the length of the sequence (x) are as in any of the following motifs:

15 K-(x)₃-L-(x)₁-K,
R-(x)₃-L-(x)₁-K,
K-(x)₁-L-(x)₃-K,
H-(x)₁-L-(x)₂-K,
R-(x)₂-W-(x)₀-R,
K-(x)₂-L-(x)₀-R,
20 R-(x)₁-V-(x)₂-H,
K-(x)₁-F-(x)₃-K,
R-(x)₀-Y-(x)₂-K or
R-(x)₂-I-(x)₂-K

25 Further, it is preferred that at least one of the residues of the sequence (x) is a basic amino acid residue, hydrophilic amino acid residue or G. In one preferred embodiment it may be a basic residue selected from K, R or H, wherein H is more preferred. In another preferred embodiment, it may be a hydrophilic residue selected from either charged or uncharged hydrophilic residues, such as D, E, H, K, N, Q, R, S or T, wherein H is more preferred among the charged amino acid residues, and Q or S are more preferred among the uncharged residues. In still another preferred embodiment the residue may be G.

35 According to the invention, the amino acid motif as defined above is a FGFR binding amino acid motif. The present invention provides the following non-limited examples

of such FGFR binding amino acid motifs:

KWFSLGK (SEQ ID NO: 8)

RYQWR (SEQ ID NO: 9)

KGHLR (SEQ ID NO: 10)

5 RHVHSH (SEQ ID NO: 11)

RFHILFK (SEQ ID NO: 12)

KALPEGK (SEQ ID NO: 13)

HHLAVK (SEQ ID NO: 14).

10 According to the invention any of the above listed amino acid sequences is capable of binding FGFR and this capability is conferred to a compound comprising said sequence. Accordingly, a compound comprising any of the above sequences is concerned as a promising compound for the purpose of activating FGFR. Among
15 such promising compounds of the invention are preferred peptide sequences which comprise at most 105 amino acid residues.

Thus, according to the invention, one of the preferred compounds is a compound that comprises an isolated peptide sequence which comprises a FGFR binding motif selected from the motifs identified above (SEQ ID NOs: 8-14), wherein the isolated
20 peptide sequence, preferably, comprises an amino acid sequence selected from the following amino acid sequences:

APEKWFSLGKV (SEQ ID NO: 1),

DWNAPQIQRYQWR (SEQ ID NO: 2),

DLAQVKGHLRGYN (SEQ ID NO: 3),

25 RHVHSHMVPAN (SEQ ID NO: 4),

RFHILFKALPEGKVSPD (SEQ ID NO: 5) or

LHHLAVKTNGTG (SEQ ID NO: 6).

More preferred a compound that essentially comprises an amino acid sequence
30 selected from SEQ ID NOs: 1-6. The term "essentially comprising" means in the present context that a sequence selected from SEQ ID NOs:1-6 constitutes at least 10 % of the compound content which may be expressed either as the compound molecular mass or as the range of amino acid residues or amino acid sequences in the compound. When a selected amino acid sequence constitutes of 100% of the
35 compound content, it is understood that the compound is consisting of said se-

quence.

A compound consisting any of the sequences selected from SEQ ID NOs:1-6 are also among preferred compounds of the invention.

5 In one embodiment, a preferred compound, which consists of a sequence selected from the sequences of SEQ ID Nos:1-6, is a compound consisting of SEQ ID NO: 1. In another embodiment, a preferred compound may consist of SEQ ID NO: 2. In still another embodiment, a preferred compound may consist of SEQ ID NO: 3. Yet, a preferred compound may consists of SEQ ID NO: 4. In another preferred embodi-
10 ments the compound may consist of SEQ ID NO: 5 or SEQ ID NO: 6. In these embodiments SEQ ID NOs:1-6 are meant to be isolated peptide sequences.

An isolated peptide sequence is according to the invention an amino acid sequence of a desirable length which is produced by use of any recombinant technology
15 methods or chemical synthesis, or it was separated from a longer polypeptide or protein by a method of enzymatic or chemical cleavage

Thus, accordingly, an isolated peptide sequence may be a fragment of a protein being separated from the other parts of the protein, e.g. other fragments of the pro-
20 tein polypeptide. The present invention relates to isolated peptide fragments of the proteins which comprise a FGFR motif described above. According to the invention an isolated peptide fragment of the invention may derive from any protein, however, a preferred isolated protein fragment of the invention is derived from neural cell adhesion molecule L1. Thus, a compound of the invention may comprise or consist of
25 a peptide fragment of L1. The invention relates to any mammal L1 protien, such as for example as the identified in Swiss-Prot database polypeptides P32003, P11627, Q05695. Preferably L1 is human which has the sequence identified in Swiss-Prot database as P32004.

30 The invention in particular relates to peptide fragments of L1 which are derived from a part of L1 polypeptide which corresponds to Fibronectin type-3 modules 1 to 5 (F3, 1-5). Thus, in one embodiment a peptide fragment of L1 of the invention consists of the F3, 1 module of L1 and has the sequence identified as SEQ ID NO: 15. In another embodiment, the L1-derived peptide ftadment consists of the F3, 2 module of
35 L1 and has the sequence identified as SEQ ID NO: 16. In still another embodiment,

the fragment is the F3, 3 module of L1 and has the sequence identified as SEQ ID NO: 17. Yet, in another embodiment, the fragment is the F3, 4 module of L1 and has the sequence identified as SEQ ID NO: 18. In another embodiment the fragment may consist of the F3, 5 module of L1 and has the sequence identified as SEQ ID NO: 19.

The invention preferably relates to L1 F3,1-F3,5 modules having the following sequences:

F3,1:

PGPVPRVLSDLHLLTQSQVRVSWSPAEDHNAPIEKYDIEFEDKEMAPEKWYSLG
KVPGNQSTSTTLKLSPYVHYTFRVTAINKYGPGEPSPVSETV

F3,2: PEKNPVDVKGEGNETTNMVTWKPLRWMDWNAPQVQYRVQWRPQGTR
GPWQEQIVSD PFLVVSNTSTFVPYEIKVQA VNSQGKGPEP QVTIGYS

F3,3:

PQAIPELEGIEILNSSAVLVKWRPVDLAQVKGHLRGYNVTYWREGSQRKHSKRHH
KDHVVVPANTTSVILSGLRPYSSYHLEVQAFNGRSGPASEFT FST

F3,4:

PEGVPGHPEALHLECQSNTSLLLRLWQPPLSHNGVLTGYVLSYHPLDEGGKGQLSF
NLRDPELRTHNLTDLSPLRYRFQLQATTKEGPGEAIVREGGT

F3,5:

GISDFGNISATAGENYSVSWVPKEGQCENFRFHILFKALGEEKGGASLSPQYVSYN
QSSYTQWDLQPDTDYEIHLFKERMFRHQMAVKTNGTG

However, in some preferred embodiments of the invention it may be advantageous to use shorter peptide sequences. Thus, the invention concern fragments of the F3 modules of L1, such as fragments of the F3,1, F3,2, F3,3, F3, 4 or F3, 5 modules. It is preferred that the fragments of the latter modules comprise at least one of the sequences selected from SEQ ID NOs:1-6 or fragments of said sequences, such as fragments having the length of 5-7 amino acid residues and comprising the FGFR binding motif discussed above, for example a sequence selected from SEQ ID NOs: 8-14.

1.1 Length of peptide sequence

According to the invention, a compound of the invention comprises an amino acid
5 sequence comprising a FGFR binding motif of at most 105 amino acid residues.

Thus, in one embodiment, the compound may comprise a peptide sequence
comprising 3-105 amino acid residues, for example 3-100 amino acid residues, such
as 3-95 amino acid residues, for example 3-90 amino acid residues, such as 3-85
10 amino acid residues, for example 3-80 amino acid residues, such as 3-75 amino
acid residues, for example 3-70 amino acid residues, such as 3-65 amino acid
residues, for example 3-60, such as 3-55 amino acid residues, for example 3-50
amino acid residues, such as 3-45 amino acid residues, for example 3-40 amino
acid residues, such as 3-35 amino acid residues, such as 3-30 amino acid residues,
15 for example 3-25 amino acid residues, such as 3-20 amino acid residues, for
example 3-15, such as 3-10 amino acid residues, for example 3-5 amino acid
residues.

In another embodiment a peptide sequence of the compound may have the length
20 of between 4-100 amino acid residues, such as 4-90 amino acid residues, for
example 4-80 amino acid residues, such as 4-70, for example 4-60, such as 4-50
amino acid residues, for example 4-40 amino acid residues, such as 4-30 amino
acid residues, for example 4-20 amino acid residues, such as 4-15 amino acid
residues or 4-10.

25 In further embodiment a peptide sequence may have the length between 5 and 100
amino acid residues, such as 5 to 90 amino acid residues, for example 5 to 80
amino acid residues, such as 5 to 70, for example 5 to 60, such as 5 to 50 amino
acid residues, for example 5 to 40 amino acid residues, such as 5 to 30 amino acid
30 residues, for example 5 to 20 amino acid residues, such as 5 to 15 amino acid
residues, for example 5 to 10 amino acid residues.

In yet further embodiment the sequence may be of 6-100 amino acid residues, such
as 6-90 amino acid residues, for example 6-80 amino acid residues, such as 6-70,
35 for example 6-60, such as 6-50 amino acid residues, for example 6-40 amino acid

residues, such as 6-30 amino acid residues, for example 6-20 amino acid residues, such as 6-15 amino acid residues, for example 6-10 amino acid residues.

5 The invention also relates to amino acid sequences, which have the length of between 7-100 amino acid residues, such as 7-90 amino acid residues, 7-80 amino acid residues, such as 7-70, for example 7-60, such as 7-50 amino acid residues, for example 7-40 amino acid residues, such as 7-30 amino acid residues, for example 7-20 amino acid residues, such as 7-15 amino acid residues, for example 8, 9, 10, 11, 12, 13, or 14 amino acid residues.

10

The length of the isolated sequences may also be of 8-80 amino acid residues, such as 8-70, for example 8-60, such as 8-50 amino acid residues, for example 8-40 amino acid residues, such as 8-30 amino acid residues, for example 8-20 amino acid residues. Or it may be of 9-80 amino acid residues, such as 9-70 amino acid residues, for example 9-60 amino acid residues, such as 9-50 amino acid residues, for example 9-40 amino acid residues, such as 9-30 amino acid residues, for example 9-20 amino acid residues.

15

A compound which comprises an amino acid sequence which is of 10-80 amino acid residues, such as 10-70 amino acid residues, for example 10-60 amino acid residues, such as 10-50 amino acid residues, for example 10-40 amino acid residues, such as 10-30 amino acid residues, for example 10-20 amino acid residues is also within the scope of the invention.

20

25

In one preferred embodiment of the invention the minimal length of an amino acid sequences of the compound be of 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 amino acid residues, and the maximal is 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50 amino acid residues. A compound comprising an amino acid sequence the length of which is in the range of 25 - 36 amino acid residues is also preferred.

30

Still, more preferred compounds are those that comprise isolated peptide sequences the length of which is at most 20 amino acid residues. The compounds comprising individual peptide fragments having the length of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18 amino acid residues are among most preferred compounds.

35

It is understood that all the above discussed peptide sequences comprise either an FGFR binding motif of the invention or a peptide fragment of L1 described above, and are capable of binding to FGFR.

5 **1.3. Multimeric compound**

10 An isolated peptide sequence of the invention may be connected to another isolated peptide sequence by a chemical bond in a fusion protein with the proviso that the fusion protein is not L1 protein. Two or more isolated sequences of the invention may also be connected to each other through a linker grouping making other types of multimeric presentation of peptide sequences of the invention. Both the latter fusion proteins and multimers are concerned as compounds of the invention.

15 Thus, a peptide sequence of the invention may in some embodiments be formulated as a polymer comprising several copies of said peptide sequence. The polymers comprising two sequences are termed herein as dimers. The polymers comprising four peptide sequences are termed herein as tetramers.

20 According to the invention a multimeric compound may be a polymer of two or more identical or different peptide sequences of the invention, wherein in a preferred embodiment, one of the two or more amino acid sequences is selected from SEQ ID NOs:1-6, or fragments or variants of said sequences.

25 In some embodiments, the compound may comprise two identical amino acid sequences selected from SEQ ID NOs:1-6, or two identical fragments or variants of the selected sequence, wherein said amino acid sequences, fragments or variants. Or, the compound may comprise four identical copies of an amino acid sequence selected from SEQ ID NOs:1-6, or four identical fragments or variants of the selected sequence.

30 In other embodiments, the compound may comprise two or more different amino acid sequences, wherein at least one of the two amino acid sequences is a sequence selected from SEQ ID NOs: 1-6, or fragments or variants thereof.

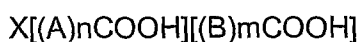
35 In yet other embodiments, the compound may comprise two or more different amino

acid sequences, wherein said two or more amino acid sequences are selected from SEQ ID NO: 1-6, or fragments or variants thereof.

5 A preferred multimeric compound of the invention is a compound wherein the amino acid sequences are connected to each other through a linker or a linker grouping.

A linker is according to the invention may be any molecule or chemical moiety capable of cross-linking two or more peptide sequences, for example it may be an achiral di-, tri- or tetracarboxylic acid of the general formula

10



wherein

n and m independently are an integer of from 1 to 20,

15 X is HN, $\text{H}_2\text{N}(\text{CR}_2)_p\text{CR}$, $\text{RHN}(\text{CR}_2)_p\text{CR}$, $\text{HO}(\text{CR}_2)_p\text{CR}$, $\text{HS}(\text{CR}_2)_p\text{CR}$, halogen- $(\text{CR}_2)_p\text{CR}$, $\text{HOOC}(\text{CR}_2)_p\text{CR}$, $\text{ROOC}(\text{CR}_2)_p\text{CR}$, $\text{HCO}(\text{CR}_2)_p\text{CR}$, $\text{RCO}(\text{CR}_2)_p\text{CR}$, $[\text{HOOC}(\text{A})_n][\text{HOOC}(\text{B})_m]\text{CR}(\text{CR}_2)_p\text{CR}$, $\text{H}_2\text{N}(\text{CR}_2)_p$, $\text{RHN}(\text{CR}_2)_p$, $\text{HO}(\text{CR}_2)_p$, $\text{HS}(\text{CR}_2)_p$, halogen- $(\text{CR}_2)_p$, $\text{HOOC}(\text{CR}_2)_p$, $\text{ROOC}(\text{CR}_2)_p$, $\text{HCO}(\text{CR}_2)_p$, $\text{RCO}(\text{CR}_2)_p$, or $[\text{HOOC}(\text{A})_n][\text{HOOC}(\text{B})_m](\text{CR}_2)_p$, wherein p is 0 or integer of from 1 to 20,

20 A and B independently are a substituted or unsubstituted C_{1-10} alkyl, a substituted or unsubstituted C_{2-10} alkenyl, a substituted or unsubstituted cyclic moiety, a substituted or unsubstituted heterocyclic moiety, a substituted or unsubstituted aromatic moiety, or A and B together form a substituted or unsubstituted cyclic moiety, substituted or unsubstituted heterocyclic moiety, substituted or unsubstituted aromatic moiety.

25

Under the term C_{1-10} alkyl is meant straight or branched chain alkyl groups having 1-10 carbon atoms, e.g. methyl, ethyl, isopropyl, butyl, and tertbutyl.

Under the term C_{2-10} alkenyl is meant straight or branched chain alkenyl groups having 2-10 carbon atoms, e.g. ethynyl, propenyl, isopropenyl, butenyl, and tert-butenyl.

30

Under the term cyclic moiety is meant cyclohexan, and cyclopentane.

Under the term aromatic moiety is meant phenyl.

35

The wording "A and B forms a cyclic, heterocyclic or aromatic moiety" denotes cyclohexan, piperidine, benzene, and pyridine.

5 In those embodiments where a multimeric compound of the invention comprises the linker of above, the compound is preferably obtained by the LPA method (a ligand presentation assembly method) as described in WO0018791 and WO2005014623.

10 Another example of a preferred linker of the invention may be amino acid lysine. Individual peptide sequences may be attached to a core molecule such as lysine forming thereby a dendritic multimer (dendrimer) of an individual peptide sequence(s). Production of dendrimers is also well known in the art (PCT/US90/02039, Lu et al., (1991) Mol Immunol. 28:623-630; Defoort et al., (1992) Int J Pept Prot Res. 40:214-221; Drijfhout et al. (1991) Int J Pept Prot Res. 37:27-32), and dedrimers are at present widely used in research and in medical
15 applications.

Still, in some embodiments, amino acid cystein may be preferred a linker molecule.

20 One of the referred embodiments of the invention concerns a compound comprising four individual amino acid sequences attached to the lysine core molecule, a dendritic tetramer/dendrimer of a peptide sequence of the invention.

25 Multimeric compounds of the invention, such as LPA-dimers or dendrimers, are most preferred compounds of the invention. However, other types of multimeric compounds comprising two or more individual sequences of the invention are also in the scope of the invention. These compounds may be produced using technologies known in the art.

30 The peptide sequences may be covalently bound to the linker through their amino- or carboxy-groups, preferably through the N- or C terminal amino- or carboxy-groups.

1.4. Fragment and variant of peptide sequence

35 In some embodiments of the invention a compound may comprise a variant or a

fragment of an FGFR binding sequences of the invention, preferably a fragment or variant of a sequence selected from SEQ ID NOs:1-6.

According to the invention, a variant of an amino acid sequence selected from the sequences SEQ ID NOs: 1-6 may be

- i) an amino acid sequence which has at least 60% identity with a selected sequence, such as 61-65% identity, for example 66-70% identity, such as 71-75% identity, for example 76-80% identity, such as 81-85 % identity, for example 86-90% identity, such as 91-95% identity, for example 96-99% identity, wherein the identity is defined as a percentage of identical amino acids in said sequence when it is collated with the selected sequence. The identity between amino acid sequences may be calculated using well known algorithms such as BLOSUM 30, BLOSUM 40, BLOSUM 45, BLOSUM 50, BLOSUM 55, BLOSUM 60, BLOSUM 62, BLOSUM 65, BLOSUM 70, BLOSUM 75, BLOSUM 80, BLOSUM 85, or BLOSUM 9;
- ii) an amino acid sequence which has at least 60% positive amino acid matches with a selected sequence, such as 61-65% positive amino acid matches, for example 66-70% positive amino acid matches, such as 71-75% positive amino acid matches, for example 76-80% positive amino acid matches, such as 81-85 % positive amino acid matches, for example 86-90% positive amino acid matches, such as 91-95% positive amino acid matches, for example 96-99% positive amino acid matches, wherein the positive amino acid match is defined as the presence at the same position in two compared sequences of amino acid residues which has similar of physical and/or chemical properties. Preferred positive amino acid matches of the present invention are K to R, E to D, L to M, Q to E, I to V, I to L, A to S, Y to W, K to Q, S to T, N to S and Q to R;
- iii) an amino acid sequence which is identical to a selected sequence, or it has at least 60% identity with said sequence such as 61-65% identity, for example 66-70% identity, such as 71-75% identity, for example 76-80% identity, such as 81-85 % identity, for example 86-90% identity, such as 91-95% identity, for example 96-99% identity, or has at least 60% posi-

5 tive amino acid matches with the selected sequence, such as 61-65% positive amino acid matches, for example 66-70% positive amino acid matches, such as 71-75% positive amino acid matches; for example 76-80% positive amino acid matches, such as 81-85 % positive amino acid matches, for example 86-90% positive amino acid matches, such as 91-95% positive amino acid matches, for example 96-99% positive amino acid matches and comprises other chemical moieties, e. g. phosphoryl, sulphur, acetyl, glycosyl moieties.

10 In one aspect the term "variant of a peptide sequence" means that the peptide sequence may be modified, for example by substitution of one or more of the amino acid residues. Both L-amino acids and D-amino acids may be used. Other modification may comprise derivatives such as esters, sugars, etc. Examples are methyl and acetyl esters.

15 In another aspect, variants of the peptide fragments according to the invention may comprise, within the same variant, or fragments thereof or among different variants, or fragments thereof, at least one substitution, such as a plurality of substitutions introduced independently of one another. Variants of the complex, or fragments thereof may thus comprise conservative substitutions independently of one another, wherein at least one glycine (Gly) of said variant, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Ala, Val, Leu, and Ile, and independently thereof, variants, or fragments thereof, wherein at least one alanine (Ala) of said variants, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Val, Leu, and Ile, and independently thereof, variants, or fragments thereof, wherein at least one valine (Val) of said variant, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Ala, Leu, and Ile, and independently thereof, variants, or fragments thereof, wherein at least one leucine (Leu) of said variant, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Ala, Val, and Ile, and independently thereof, variants, or fragments thereof, wherein at least one isoleucine (Ile) of said variants, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Ala, Val and Leu, and independently thereof, variants, or fragments thereof wherein at least one aspartic acids (Asp) of said vari-

20

25

30

35

ant, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Glu, Asn, and Gln, and independently thereof, variants, or fragments thereof, wherein at least one asparagine (Asn) of said variants, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Asp, Glu, and Gln, and independently thereof, variants, or fragments thereof, wherein at least one glutamine (Gln) of said variants, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Asp, Glu, and Asn, and wherein at least one phenylalanine (Phe) of said variants, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Tyr, Trp, His, Pro, and preferably selected from the group of amino acids consisting of Tyr and Trp, and independently thereof, variants, or fragments thereof, wherein at least one tyrosine (Tyr) of said variants, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Phe, Trp, His, Pro, preferably an amino acid selected from the group of amino acids consisting of Phe and Trp, and independently thereof, variants, or fragments thereof, wherein at least one arginine (Arg) of said fragment is substituted with an amino acid selected from the group of amino acids consisting of Lys and His, and independently thereof, variants, or fragments thereof, wherein at least one lysine (Lys) of said variants, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Arg and His, and independently thereof, variants, or fragments thereof, and independently thereof, variants, or fragments thereof, and wherein at least one proline (Pro) of said variants, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Phe, Tyr, Trp, and His, and independently thereof, variants, or fragments thereof, wherein at least one cysteine (Cys) of said variants, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Asp, Glu, Lys, Arg, His, Asn, Gln, Ser, Thr, and Tyr.

It thus follows from the above that the same functional equivalent of a peptide fragment, or fragment of said functional equivalent may comprise more than one conservative amino acid substitution from more than one group of conservative amino acids as defined herein above. The term "conservative amino acid substitution" is used synonymously herein with the term "homologous amino acid substitution".

The groups of conservative amino acids are as the following:

A, G (neutral, weakly hydrophobic),

Q, N, S, T (hydrophilic, non-charged)
E, D (hydrophilic, acidic)
H, K, R (hydrophilic, basic)
L, P, I, V, M, F, Y, W (hydrophobic, aromatic)
5 C (cross-link forming)

Conservative substitutions may be introduced in any position of a preferred predetermined peptide of the invention or fragment thereof. It may however also be desirable to introduce non-conservative substitutions, particularly, but not limited to, a
10 non-conservative substitution in any one or more positions.

A non-conservative substitution leading to the formation of a functionally equivalent fragment of the peptide of the invention would for example differ substantially in polarity, for example a residue with a non-polar side chain (Ala, Leu, Pro, Trp, Val, Ile,
15 Leu, Phe or Met) substituted for a residue with a polar side chain such as Gly, Ser, Thr, Cys, Tyr, Asn, or Gln or a charged amino acid such as Asp, Glu, Arg, or Lys, or substituting a charged or a polar residue for a non-polar one; and/or ii) differ substantially in its effect on peptide backbone orientation such as substitution of or for Pro or Gly by another residue; and/or iii) differ substantially in electric charge, for
20 example substitution of a negatively charged residue such as Glu or Asp for a positively charged residue such as Lys, His or Arg (and vice versa); and/or iv) differ substantially in steric bulk, for example substitution of a bulky residue such as His, Trp, Phe or Tyr for one having a minor side chain, e.g. Ala, Gly or Ser (and vice versa).

25 Substitution of amino acids may in one embodiment be made based upon their hydrophobicity and hydrophilicity values and the relative similarity of the amino acid side-chain substituents, including charge, size, and the like

According to the invention a fragment of a selected sequence of the invention, such
30 as a sequence selected from SEQ ID NOs:1-6, may be an amino acid sequence, which has about 25 - 99 % of the length of the selected amino acid sequence.

Both fragments and variants of selected sequences of the invention are according to the invention functional homologues of said selected sequences.

By the term "functional homologue" of a selected amino acid sequence is in the present context meant a molecule which is capable of one or more biological functions of the selected sequence or a compound comprising said selected sequence, in a preferred embodiment biological functions, which are executed through the mechanism of binding and activating FGFR.

1.5 Biological activity

According to the invention, compounds described above are functionally active compounds. The compounds are capable of binding to a functional cell-surface receptor and activating the receptor. The receptor according to the invention is FGFR and may be selected from the group of FGFR receptors comprising Fibroblast Growth Factor Receptor 1 (FGFR1), Fibroblast Growth Factor Receptor 2 (FGFR2), Fibroblast Growth Factor Receptor 3 (FGFR3), Fibroblast Growth Factor Receptor 4 (FGFR4) or Fibroblast Growth Factor Receptor 5 (FGFR5). The above compound according to the invention is capable of binding to any of the latter FGFRs at a binding site located in the extracellular part of the receptor, such as a binding site located in the Ig1, Ig2 and/or Ig3 domains of FGFR, preferably in the Ig2 and/or Ig3 domains of FGFR. According to the invention the latter binding site is different from the other described binding sites of FGFR (e.g. see: Plotnikov AN, Hubbard SR, Schlessinger J, Mohammadi M..Cell. 2000 May 12;101(4):413-24).

Thus, the invention relates to a functional cell-surface receptor. By "functional cell-surface receptor" is meant that a receptor is located in the outer plasma membrane of the cell and has an identifiable group of extracellular ligands. Binding of these ligands to the receptor induces intracellular signal transduction, which results in a physiological response of the cell. The physiological response, such as for example the ligand binding induced change in cell metabolism, induction of cell differentiation, termination or induction of cell proliferation, survival or death of the cell, change in motile behavior of the cell, depends on the nature of receptor ligand, the receptor cellular and extracellular environments and/or particularity of the ligand-receptor interaction, e.g. affinity and/or duration of interaction. Ligand binding to a functional receptor normally results in a change in the activation status of the receptor, such as the receptor becoming capable of initiating a cascade of biochemical reactions inside the cell resulting in one of the above mentioned physiological responses of the

cell collectively termed "receptor signalling" or "signal transduction". Binding of a ligand may also result in inhibiting the receptor activity which means that the receptor is becoming "silent" or "inactive" and is not any more capable of initiating a cascade of biochemical reactions which it normally does due to the ligand binding. The invention relates to both compounds which are capable of activating FGFR and compounds which are capable of inhibiting FGFR. In one preferred embodiment the invention relates to compounds that are capable of activating FGFR, in another preferred embodiment the invention relates to compounds that are capable of inhibiting FGFR. Preferably, the invention relates to compounds which are capable of activating FGFR 1 and stimulating FGFR 1 signalling.

Activation of a receptor often is preceded by the receptor dimerisation induced by ligand binding. Therefore, a capability of the compound to promote receptor dimerisation (or multimerisation) is concerned by the invention as an advantageous feature. Thus, in one embodiment the invention relates to a compound that is capable of dimerising of FGFR.

Compounds of the invention may be capable of attenuating binding of other ligands to FGFR despite of occupying an alternative binding site(s). Thus, the compounds of the invention may modulate receptor signalling dependent on another ligand binding. It is known that a cellular response to the activation of a receptor depends on the strength of receptor stimulation, which may, for example, be characterised by the value of affinity of interaction of a ligand with the receptor, and/or by the duration of such interaction. Thus, both affinity and duration of interaction of FGF and the receptor may be affected by a compound of the invention. Accordingly, it is another embodiment of the invention to provide a compound, which is capable of modulating the receptor signalling induced by another receptor ligand, for example FGF or FGL-peptide of WO 03/016351.

In the present context the term "interacting" is used interchangeably with the term "binding" and refers to a direct or indirect contact between a compound of the invention and the FGF receptor, preferably a direct interaction. The term "direct interaction" means that the compound in question binds directly to the receptor.

The binding affinity of the compound according to the invention preferably has K_d value in the range of 10^{-3} to 10^{-10} M, such as preferably in the range of 10^{-4} to 10^{-8} M. According to the present invention the binding affinity may be determined by any available assays suitable for this purpose, such as for example surface plasmon resonance (SPR) analysis or nuclear magnetic resonance (NMR) spectroscopy.

The binding affinity of the compound to FGFR according to the invention can be modulated, such as enhanced or attenuated, at the presence of a nucleotide compound. In one embodiment the affinity of the interaction may be enhanced. In another embodiment the affinity of the interaction may be attenuated. According to the invention the affinity of the interaction is enhanced at the presence of a trinucleotidiphosphate, such as for example ATP, GTP or UTP, and the affinity is attenuated at the presence of a mononucleotidiphosphate, such as for example AMP, GMP, or at the presence of dinucleotidiphosphate, for example ADP or GDP. Thus, in some embodiments the compound of the invention may be used combination with a nucleotide for the purpose of optimal activating or inhibiting FGFR.

Binding of the compound of the invention to FGFR leads to a series of cellular responses mediated by FGFR. Thus, the compound which is capable of binding to FGFR and activating/inhibiting FGFR is also capable of inducing differentiation of FGFR presenting cells, modulating of proliferation of FGFR presenting cells, stimulating survival of FGFR presenting cells, and /or stimulating morphological plasticity of FGFR presenting cells, inducing angiogenesis, anti-oxidative stress and anti-inflammatory activity.

By the term "cells presenting FGFR" is meant cells expressing FGFR on the external membrane of the cells, these cells are for example neurons, glial cells, all types of muscle cells, neuroendocrine cells, gonadal cells and kidney cells, endothelial cells fibroblasts, osteoblasts, cancer, stem and embryonic cells.

In one preferred embodiment the compound is capable of stimulating neurite outgrowth.

In another preferred embodiment the compound is capable of stimulating cell survival.

In another preferred embodiment the compound is capable of stimulating synaptic plasticity. Accordingly, the compound is capable of stimulating learning and memory as well.

5

In still another preferred embodiment the compound is capable of stimulating differentiation of stem cells.

Stimulation of neural cell differentiation and plasticity

10 Substances with the potential to promote neurite outgrowth as well as stimulate regeneration and/or differentiation of neuronal cells, such as certain endogenous trophic factors, are prime targets in the search for compounds that facilitate for example neuronal regeneration and other forms of neuronal plasticity. To evaluate the potential of the present compound, the ability to stimulate the neurite outgrowth related signalling, interfere with cell adhesion, stimulate neurite outgrowth, regeneration of nerves, may be investigated.

15

Compounds of the present invention are shown to promote neurite outgrowth and are therefore considered to be good promoters of regeneration of neuronal connections, and thereby of functional recovery after damages as well as promoters of neuronal function in other conditions where such effect is required. Furthermore, compounds of the present invention are capable of stimulating neuronal progenitor cell differentiation into mature neurons. Compounds of the present invention are also potent stimulators of morphological plasticity of neurons associated with learning and memory.

20

25

In the present context "differentiation" is related both to the processes of initiation of differentiation of neuronal precursor cells, maturation of immature neurons, such as neurite outgrowth which take place after the last cell division of said neurons, and morphological plasticity of mature neurons, such as takes place in the brain in connection with learning and memory. Thus, the compounds of the present invention may be capable of stopping neural precursor and immature neural cell division and initiating maturation said cells, such as initiating extension of neurites. Otherwise, "differentiation" is related to initiation of the process of genetic, biochemical, morphological and physiological transformation of neuronal progenitor

30

35

cells, immature neural cells or embryonic stem cells leading to formation of cells having functional characteristics of normal neuronal cell as such characteristics are defined in the art. The invention defines "immature neural cell" as a cell that has at least one feature of neural cell accepted in the art as a feature characteristic for the neural cell.

According to the present invention a compound comprising at least one of the above peptide sequences is capable of stimulating neurite outgrowth. The invention concerns the neurite outgrowth improvement/stimulation such as about 75% improvement/stimulation above the value of neurite outgrowth of control/non-stimulated cells, for example 50%, such as about 150%, for example 100%, such as about 250, for example 200%, such as about 350 %, for example 300%, such as about 450%, for example 400%, such as about 500%.

Estimation of capability of a candidate compound to stimulate neurite outgrowth may be done by using any known method or assay for estimation of neurite outgrowth, such as for example as the described in Examples.

According to the invention a compound has neuritogenic activity both as an insoluble immobile component of cell growth substrate and as a soluble component of cell growth media. In the present context "immobile" means that the compound is bound/attached to a substance which is insoluble in water or a water solution and thereby it becomes insoluble in such solution as well. For medical applications both insoluble and soluble compounds are considered by the application, however soluble compounds are preferred. Under "soluble compound" is understood a compound, which is soluble in water or a water solution.

Antibody

It is an objective of the present invention to provide an antibody, antigen binding fragment or recombinant protein thereof capable of recognizing and selectively binding to an epitope comprising an FGFR motif of the invention, such as epitope comprising a sequence selected from SEQ ID NOs: 8-14 or a sequence selected from SEQ ID NOs:1-6, or a fragment of said sequence, preferably an epitope in L1 polypeptide.

By the term "epitope" is meant the specific group of atoms (on an antigen molecule) that is recognized by (that antigen's) antibodies (thereby causing an immune response). The term "epitope" is the equivalent to the term "antigenic determinant".

5 The epitope may comprise 3 or more amino acid residues, such as for example 4, 5, 6, 7, 8 amino acid residues, located in close proximity, such as within a contiguous amino acid sequence, or located in distant parts of the amino acid sequence of an antigen, but due to protein folding have been approached to each other.

10 Antibody molecules belong to a family of plasma proteins called immunoglobulins, whose basic building block, the immunoglobulin fold or domain, is used in various forms in many molecules of the immune system and other biological recognition systems. A typical immunoglobulin has four polypeptide chains, containing an antigen binding region known as a variable region and a non-varying region known as
15 the constant region.

Native antibodies and immunoglobulins are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies between the heavy chains of
20 different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (VH) followed by a number of constant domains. Each light chain has a variable domain at one end (VL) and a constant domain at its other end. The constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light and heavy chain variable domains (Novotny J, & Haber E. Proc Natl Acad Sci U S A. 82(14):4592-6, 1985).
25

30 Depending on the amino acid sequences of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are at least five (5) major classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM, and several of these may be further divided into subclasses (isotypes), e.g. IgG-1, IgG-2, IgG-3 and IgG-4; IgA-1 and IgA-2. The heavy chains constant domains that correspond to
35

the different classes of immunoglobulins are called alpha (α), delta (δ), epsilon (ϵ), gamma (γ) and mu (μ), respectively. The light chains of antibodies can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino sequences of their constant domain. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

The term "variable" in the context of variable domain of antibodies, refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies. The variable domains are for binding and determine the specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed through the variable domains of antibodies. It is concentrated in three segments called complementarity determining regions (CDRs) also known as hypervariable regions both in the light chain and the heavy chain variable domains.

The more highly conserved portions of variable domains are called the framework (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a β -sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the β -sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies. The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

An antibody that is contemplated for use in the present invention thus can be in any of a variety of forms, including a whole immunoglobulin, an antibody fragment such as Fv, Fab, and similar fragments, a single chain antibody which includes the variable domain complementarity determining regions (CDR), and the like forms, all of which fall under the broad term "antibody", as used herein. The present invention contemplates the use of any specificity of an antibody, polyclonal or monoclonal, and is not limited to antibodies that recognize and immunoreact with a specific antigen. In preferred embodiments, in the context of both the therapeutic and screening methods described below, an antibody or fragment thereof is used that is immunospecific for an antigen or epitope of the invention.

The term "antibody fragment" refers to a portion of a full-length antibody, generally the antigen binding or variable region. Examples of antibody fragments include Fab, Fab', F(ab')₂ and Fv fragments. Papain digestion of antibodies produces two identical antigen binding fragments, called the Fab fragment, each with a single antigen binding site, and a residual "Fc" fragment, so-called for its ability to crystallize readily. Pepsin treatment yields an F(ab')₂ fragment that has two antigen binding fragments that are capable of cross-linking antigen, and a residual other fragment (which is termed pFc'). Additional fragments can include diabodies, linear antibodies, single-chain antibody molecules, and multispecific antibodies formed from antibody fragments. As used herein, "functional fragment" with respect to antibodies, refers to Fv, F(ab) and F(ab')₂ fragments.

The term "antibody fragment" is used herein interchangeably with the term "antigen binding fragment".

Antibody fragments may be as small as about 4 amino acids, 5 amino acids, 6 amino acids, 7 amino acids, 9 amino acids, about 12 amino acids, about 15 amino acids, about 17 amino acids, about 18 amino acids, about 20 amino acids, about 25 amino acids, about 30 amino acids or more. In general, an antibody fragment of the invention can have any upper size limit so long as it has similar or immunological properties relative to antibody that binds with specificity to an epitope comprising a peptide sequence selected from any of the sequences identified herein as SEQ ID NOs: 1-14, or a fragment of said sequences. Thus, in context of the present invention the term "antibody fragment" is identical to term "antigen binding fragment".

Antibody fragments retain some ability to selectively bind with its antigen or receptor. Some types of antibody fragments are defined as follows:

(1) Fab is the fragment that contains a monovalent antigen-binding fragment of an antibody molecule. A Fab fragment can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain.

(2) Fab' is the fragment of an antibody molecule can be obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain. Two Fab' fragments are obtained per antibody molecule.

Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region.

(3) (Fab')₂ is the fragment of an antibody that can be obtained by treating whole antibody with the enzyme pepsin without subsequent reduction.

(4) F(ab')₂ is a dimer of two Fab' fragments held together by two disulfide bonds.

Fv is the minimum antibody fragment that contains a complete antigen recognition and binding site. This region consists of a dimer of one heavy and one light chain variable domain in a tight, non-covalent association (V_H-V_L dimer). It is in this configuration that the three CDRs of each variable domain interact to define an antigen binding site on the surface of the V_H-V_L dimer. Collectively, the six CDRs confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

(5) Single chain antibody ("SCA"), defined as a genetically engineered molecule containing the variable region of the light chain, the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule. Such single chain antibodies are also referred to as "single-chain Fv" or "sFv" antibody fragments. Generally, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains that enables the sFv to form the desired structure for antigen binding. For a review of sFv see Pluckthun in The Pharmacology of Monoclonal Antibodies 113: 269-315 Rosenberg and Moore eds. Springer-Verlag, NY, 1994.

The term "diabodies" refers to a small antibody fragments with two antigen-binding sites, which fragments comprise a heavy chain variable domain (V_H) connected to a light chain variable domain (V_L) in the same polypeptide chain (V_H-V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161, and Hollinger et al., Proc. Natl. Acad. Sci. USA 90: 6444-6448 (1993).

The invention contemplate both polyclonal and monoclonal antibody, antigen binding fragments and recombinant proteins thereof which are capable of binding an epiyope according to the invention.

5 The preparation of polyclonal antibodies is well-known to those skilled in the art. See, for example, Green et al. 1992. Production of Polyclonal Antisera, in: Immunochemical Protocols (Manson, ed.), pages 1-5 (Humana Press); Coligan, et al., Production of Polyclonal Antisera in Rabbits, Rats Mice and Hamsters, in: Current Protocols in Immunology, section 2.4.1, which are hereby incorporated by reference.

10

The preparation of monoclonal antibodies likewise is conventional. See, for example, Kohler & Milstein, Nature, 256:495-7 (1975); Coligan, et al., sections 2.5.1-2.6.7; and Harlow, et al., in: Antibodies: A Laboratory Manual, page 726, Cold Spring Harbor Pub. (1988), Monoclonal antibodies can be isolated and purified from hybridoma cultures by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography. See, e.g., Coligan, et al., sections 2.7.1-2.7.12 and sections 2.9.1-2.9.3; Barnes, et al., Purification of Immunoglobulin G (IgG). In: Methods in Molecular Biology, 1992, 10:79-104, Humana Press, NY.

15

20

Methods of *in vitro* and *in vivo* manipulation of monoclonal antibodies are well known to those skilled in the art. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler and Milstein, 1975, Nature 256, 495-7, or may be made by recombinant methods, e.g., as described in US 4,816,567. The monoclonal antibodies for use with the present invention may also be isolated from phage antibody libraries using the techniques described in Clackson et al., 1991, Nature 352: 624-628, as well as in Marks et al., 1991, J Mol Biol 222: 581-597. Another method involves humanizing a monoclonal antibody by recombinant means to generate antibodies containing human specific and recognizable sequences. See, for review, Holmes, et al., 1997, J Immunol 158:2192-2201 and Vaswani, et al., 1998, Annals Allergy, Asthma & Immunol 81:105-115.

25

30

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method.

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (US 4,816,567); Morrison et al., 1984, Proc Natl Acad Sci 81: 6851-6855.

Methods of making antibody fragments are also known in the art (see for example, Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, NY, 1988, incorporated herein by reference). Antibody fragments of the present invention can be prepared by proteolytic hydrolysis of the antibody or by expression in *E. coli* of DNA encoding the fragment. Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies conventional methods. For example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted F(ab')₂. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monova-

lent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and an Fc fragment directly. These methods are described, for example, in US 4,036,945 and US 4,331,647, and references contained therein. These patents are hereby incorporated in their entireties by reference.

5

Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody. For example, Fv fragments comprise an association of V_H and V_L chains. This association may be noncovalent or the variable chains can be linked by an intermolecular disulfide bond or cross-linked by chemicals such as glutaraldehyde. Preferably, the Fv fragments comprise V_H and V_L chains connected by a peptide linker. These single-chain antigen binding proteins (sFv) are prepared by constructing a structural gene comprising DNA sequences encoding the V_H and V_L domains connected by an oligonucleotide. The structural gene is inserted into an expression vector, which is subsequently introduced into a host cell such as *E. coli*. The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing sFvs are described, for example, by Whitlow, et al., 1991, In: Methods: A Companion to Methods in Enzymology, 2:97; Bird et al., 1988, Science 242:423-426; US 4,946,778; and Pack, et al., 1993, BioTechnology 11:1271-77.

10
15
20

Another form of an antibody fragment is a peptide coding for a single complementarity-determining region (CDR). CDR peptides ("minimal recognition units") are often involved in antigen recognition and binding. CDR peptides can be obtained by cloning or constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthesize the variable region from RNA of antibody-producing cells. See, for example, Larrick, et al., Methods: a Companion to Methods in Enzymology, Vol. 2, page 106 (1991).

25
30

The invention contemplates human and humanized forms of non-human (e.g. murine) antibodies. Such humanized antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) that contain a minimal sequence derived

35

from non-human immunoglobulin, such as the epitope recognising sequence. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a nonhuman species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. Humanized antibody(es) containing a minimal sequence(s) of antibody(es) of the invention, such as a sequence(s) recognising the epitope(s) described herein, is one of the preferred embodiments of the invention.

10 In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and optimize antibody performance. In general, humanized antibodies
15 will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region
20 (Fc), typically that of a human immunoglobulin. For further details, see: Jones et al., 1986, Nature 321, 522-525; Reichmann et al., 1988, Nature 332, 323-329; Presta, 1992, Curr Op Struct Biol 2:593-596; Holmes et al., 1997, J Immunol 158:2192-2201 and Vaswani, et al., 1998, Annals Allergy, Asthma & Immunol 81:105-115.

25 The generation of antibodies may be achieved by any standard method in the art for producing polyclonal and monoclonal antibodies using natural or recombinant fragments of L1, said fragment comprising a sequence selected from SEQ ID NO: 1-14, as an antigen. Such antibodies may be also generated using variants, homologues or fragments of peptide sequences of SEQ ID NOs:1-14, said variants, homologues
30 and fragments are immunogenic peptide sequences which meet the following criteria:

- (i) being a contiguous amino acid sequence of at least 6 amino acids;
- (ii) comprising the FGFR motif of the invention.

The antibodies may also be produced *in vivo* by the individual to be treated, for example, by administering an immunogenic fragment according to the invention to said individual. Accordingly, the present invention further relates to a vaccine comprising an immunogenic fragment described above.

5

The application also relates to a method for producing an antibody of the invention said method comprising a step of providing of an immunogenic fragment described above.

10

The invention relates both to antibodies, which are capable of modulating, such as enhancing or attenuating, biological function of L1, in particular a function related to neural cell differentiation, survival and/or plasticity, and to an antibody, which can recognise and specifically bind the latter proteins without modulating biological activity thereof.

15

The invention relates to use of the above antibodies for 1) therapeutic applications when the modulation of activity of L1 or FGFR is needed, 2) detecting and/or monitoring L1 or a protein comprising the above described epitope *in vitro* and/or *in vivo* for diagnostic purposes, 3) research purposes.

20

Production of peptide sequences

The peptide sequences of the present invention may be prepared by any conventional synthetic methods, recombinant DNA technologies, enzymatic cleavage of full-length proteins which the peptide sequences are derived from, or a combination of said methods.

25

Recombinant preparation

30

Thus, in one embodiment the peptides of the invention are produced by use of recombinant DNA technologies.

35

The DNA sequence encoding a peptide or the corresponding full-length protein the peptide originates from may be prepared synthetically by established standard methods, e.g. the phosphoramidite method described by Beaucage and Caruthers, 1981, Tetrahedron Lett. 22:1859-1869, or the method described by Matthes et al.,

1984, EMBO J. 3:801-805. According to the phosphoamidite method, oligonucleotides are synthesised, e.g. in an automatic DNA synthesiser, purified, annealed, ligated and cloned in suitable vectors.

5 The DNA sequence encoding a peptide may also be prepared by fragmentation of the DNA sequences encoding the corresponding full-length protein of peptide origin, using DNAase I according to a standard protocol (Sambrook et al., Molecular cloning: A Laboratory manual. 2rd ed., CSHL Press, Cold Spring Harbor, NY, 1989). The present invention relates to full-length proteins selected from the groups of proteins
10 identified above. The DNA encoding the full-length proteins of the invention may alternatively be fragmented using specific restriction endonucleases. The fragments of DNA are further purified using standard procedures described in Sambrook et al., Molecular cloning: A Laboratory manual. 2rd ed., CSHL Press, Cold Spring Harbor, NY, 1989.

15 The DNA sequence encoding a full-length protein may also be of genomic or cDNA origin, for instance obtained by preparing a genomic or cDNA library and screening for DNA sequences coding for all or part of the full-length protein by hybridisation using synthetic oligonucleotide probes in accordance with standard techniques (cf.
20 Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor, 1989). The DNA sequence may also be prepared by polymerase chain reaction using specific primers, for instance as described in US 4,683,202 or Saiki et al., 1988, Science 239:487-491.

25 The DNA sequence is then inserted into a recombinant expression vector, which may be any vector, which may conveniently be subjected to recombinant DNA procedures. The choice of vector will often depend on the host cell into which it is to be introduced. Thus, the vector may be an autonomously replicating vector, i.e. a vector that exists as an extrachromosomal entity, the replication of which is independent
30 of chromosomal replication, e.g. a plasmid. Alternatively, the vector may be one which, when introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosome(s) into which it has been integrated.

In the vector, the DNA sequence encoding a peptide or a full-length protein should
35 be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence, which shows transcriptional activity in the host cell of choice and

may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the coding DNA sequence in mammalian cells are the SV 40 promoter (Subramani et al., 1981, Mol. Cell Biol. 1:854-864), the MT-1 (metallothionein gene) promoter (Palmiter et al., 1983, Science 222: 809-814) or the adenovirus 2 major late promoter. A suitable promoter for use in insect cells is the polyhedrin promoter (Vasudev-
5 van et al., 1992, FEBS Lett. 311:7-11). Suitable promoters for use in yeast host cells include promoters from yeast glycolytic genes (Hitzeman et al., 1980, J. Biol. Chem. 255:12073-12080; Alber and Kawasaki, 1982, J. Mol. Appl. Gen. 1: 419-434) or alcohol dehydrogenase genes (Young et al., 1982, in Genetic Engineering of Microorganisms for Chemicals, Hollaender et al, eds., Plenum Press, New York), or the TPI1 (US 4,599,311) or ADH2-4c (Russell et al., 1983, Nature 304:652-654) promoters. Suitable promoters for use in filamentous fungus host cells are, for instance, the ADH3 promoter (McKnight et al., 1985, EMBO J. 4:2093-2099) or the
10 tpiA promoter.
15

The coding DNA sequence may also be operably connected to a suitable terminator, such as the human growth hormone terminator (Palmiter et al., op. cit.) or (for fungal hosts) the TPI1 (Alber and Kawasaki, op. cit.) or ADH3 (McKnight et al., op. cit.)
20 promoters. The vector may further comprise elements such as polyadenylation signals (e.g. from SV 40 or the adenovirus 5 Elb region), transcriptional enhancer sequences (e.g. the SV 40 enhancer) and translational enhancer sequences (e.g. the ones encoding adenovirus VA RNAs).

25 The recombinant expression vector may further comprise a DNA sequence enabling the vector to replicate in the host cell in question. An example of such a sequence (when the host cell is a mammalian cell) is the SV 40 origin of replication. The vector may also comprise a selectable marker, e.g. a gene the product of which complements a defect in the host cell, such as the gene coding for dihydrofolate reductase
30 (DHFR) or one which confers resistance to a drug, e.g. neomycin, hydromycin or methotrexate.

The procedures used to ligate the DNA sequences coding the peptides or full-length proteins, the promoter and the terminator, respectively, and to insert them into suitable vectors containing the information necessary for replication, are well known to
35 persons skilled in the art (cf., for instance, Sambrook et al., op.cit.).

To obtain recombinant peptides of the invention the coding DNA sequences may be usefully fused with a second peptide coding sequence and a protease cleavage site coding sequence, giving a DNA construct encoding the fusion protein, wherein the protease cleavage site coding sequence positioned between the HBP fragment and second peptide coding DNA, inserted into a recombinant expression vector, and expressed in recombinant host cells. In one embodiment, said second peptide selected from, but not limited by the group comprising glutathion-S-reductase, calf thymosin, bacterial thioredoxin or human ubiquitin natural or synthetic variants, or peptides thereof. In another embodiment, a peptide sequence comprising a protease cleavage site may be the Factor Xa, with the amino acid sequence *IEGR*, enterokinase, with the amino acid sequence *DDDDK*, thrombin, with the amino acid sequence *LVPR/GS*, or *Acharombacter lyticus*, with the amino acid sequence *XXK*, cleavage site.

The host cell into which the expression vector is introduced may be any cell which is capable of expression of the peptides or full-length proteins, and is preferably a eukaryotic cell, such as invertebrate (insect) cells or vertebrate cells, e.g. *Xenopus laevis* oocytes or mammalian cells, in particular insect and mammalian cells. Examples of suitable mammalian cell lines are the HEK293 (ATCC CRL-1573), COS (ATCC CRL-1650), BHK (ATCC CRL-1632, ATCC CCL-10) or CHO (ATCC CCL-61) cell lines. Methods of transfecting mammalian cells and expressing DNA sequences introduced in the cells are described in e.g. Kaufman and Sharp, J. Mol. Biol. 159, 1982, pp. 601-621; Southern and Berg, 1982, J. Mol. Appl. Genet. 1:327-341; Loyer et al., 1982, Proc. Natl. Acad. Sci. USA 79: 422-426; Wigler et al., 1978, Cell 14:725; Corsaro and Pearson, 1981, in Somatic Cell Genetics 7, p. 603; Graham and van der Eb, 1973, Virol. 52:456; and Neumann et al., 1982, EMBO J. 1:841-845.

Alternatively, fungal cells (including yeast cells) may be used as host cells. Examples of suitable yeast cells include cells of *Saccharomyces spp.* or *Schizosaccharomyces spp.*, in particular strains of *Saccharomyces cerevisiae*. Examples of other fungal cells are cells of filamentous fungi, e.g. *Aspergillus spp.* or *Neurospora spp.*, in particular strains of *Aspergillus oryzae* or *Aspergillus niger*. The use of *Aspergillus spp.* for the expression of proteins is described in, e.g., EP 238 023.

The medium used to culture the cells may be any conventional medium suitable for growing mammalian cells, such as a serum-containing or serum-free medium containing appropriate supplements, or a suitable medium for growing insect, yeast or fungal cells. Suitable media are available from commercial suppliers or may be prepared according to published recipes (e.g. in catalogues of the American Type Culture Collection).

The peptides or full-length proteins recombinantly produced by the cells may then be recovered from the culture medium by conventional procedures including separating the host cells from the medium by centrifugation or filtration, precipitating the proteinaceous components of the supernatant or filtrate by means of a salt, e.g. ammonium sulphate, purification by a variety of chromatographic procedures, e.g. HPLC, ion exchange chromatography, affinity chromatography, or the like.

Synthetic preparation

The methods for synthetic production of peptides are well known in the art. Detailed descriptions as well as practical advice for producing synthetic peptides may be found in Synthetic Peptides: A User's Guide (Advances in Molecular Biology), Grant G. A. ed., Oxford University Press, 2002, or in: Pharmaceutical Formulation: Development of Peptides and Proteins, Frokjaer and Hovgaard eds., Taylor and Francis, 1999.

Peptides may for example be synthesised by using Fmoc chemistry and with Acm-protected cysteins. After purification by reversed phase HPLC, peptides may be further processed to obtain for example cyclic or C- or N-terminal modified isoforms. The methods for cyclization and terminal modification are well-known in the art and described in detail in the above-cited manuals.

In a preferred embodiment the peptide sequences of the invention are produced synthetically, in particular, by the Sequence Assisted Peptide Synthesis (SAPS) method.

Sequence Assisted Peptide Synthesis (SAPS)

Peptides may be synthesised either batchwise in a polyethylene vessel equipped with a polypropylene filter for filtration or in the continuous-flow version of the polyamide solid-phase method (Dryland, A. and Sheppard, R.C., (1986) J.Chem. Soc. Perkin Trans. I, 125 - 137.) on a fully automated peptide synthesiser using 9-fluorenylmethyloxycarbonyl (Fmoc) or tert. -Butyloxycarbonyl, (Boc) as N- α -amino protecting group and suitable common protection groups for side-chain functionality's.

The following is a list of chemicals and a description of the procedure that may be helpful when using SAPS for the synthesis of the peptide fragments of the invention.

3. Medicament

FGFR and L1 are known to be involved in a number of body processes in normal conditions and in disease, in particular in the neural system. These processes include differentiation, proliferation, survival, plasticity and motility of cells.

Cell death plays a key role in normal neuronal development, where 50% of the developing neurons are eliminated through programmed cell death, and in the pathophysiology of neurodegenerative conditions, such as Alzheimer's and Parkinson's diseases. FGFRs and L1 have been shown to be important determinants of neuronal survival both during development and during adulthood (Haspel et al. (2000) J Neurobiol 15:287-302; Roonprapurt et al. (2003) J Neurotrauma 20:871-882; Wiencken-Barger et al. Cereb Cortex (2004) 14:121-131; Loers et al. (2005) J Neurochem 92:1463-1476; Reuss and von Bohlen und Halbach (2003) Cell tissue Res, 313:139-57). Therefore, a compound, which is capable to promote neuronal cell survival by binding and activation FGFR is highly desirable. Thus, in one aspect the invention features compounds that promote survival of neural cells and can be used as medicaments for the treatment of conditions involving neural cell death. However, a compound of the invention may also be used as a medicament for promotion of survival of another type of cells, e.g. different type of muscle cells, or, alternatively, for promotion of cell death of still another type of cells, e.g. cancer cells, as the FGFR signalling has been shown to be a survival factor for both muscle and cancer cells (Ozen et al. (2001) J Nat Cancer Inst. 93:1783-90; Miyamoto et al. (1998) J Cell Physiol. 177:58-67; Detillieux et al. (2003) Cardiovasc Res. 57:8-19).

Activity of cell-surface receptors is strictly regulated in a healthy organism. Mutations, abnormal expression or processing of a receptor or the receptor ligands lead to abnormalities in activity of the receptor and therefore lead to dysfunction of the receptor. The dysfunction of the receptor is in turn a reason for dysfunction of the cells which use the receptor for induction and/or maintenance of various cellular processes. The latter is the manifestation of a disease. It has also been shown that attenuation of FGFR signalling leads to development of a number of different pathologic conditions, e.g. diabetes (Hart et al., Nature 2000, 408:864-8). Activation of FGF receptors is involved in normal, as well as in pathologic angiogenesis (Slavin, Cell Biol Int 1995, 19:431-44). It is important for development, proliferation, functioning and survival skeletal muscle cells, cardiomyocytes and neurons (Merle et al., J Biol Chem 1995, 270:17361-7; Cheng and Mattson, Neuron 1991, 7:1031-41; Zhu et al., Mech Ageing Dev 1999, 108:77-85). It plays a role in maintenance of normal kidney structure (Cancilla et al., Kidney Int 2001, 60:147-55), and it is implicated in wound healing and cancer disease (Powers et al., Endocr Relat Cancer. 2000, 7:165-97).

The present invention provides compounds capable of modulation of the activity of FGFRs. Consequently, said compounds are concerned by the invention as medicament for the treatment of diseases, wherein modulation of the activity of FGFRs may be considered as an essential condition for the curing.

Thus, the medicament of the invention is in one embodiment for prevention and/or treatment of

- 1) diseases and conditions of the central and peripheral nervous system, or of the muscles or of various organs, and/or
- 2) diseases or conditions of the central and peripheral nervous system, such as postoperative nerve damage, traumatic nerve damage, impaired myelination of nerve fibers, postischaemic damage, e.g. resulting from a stroke, Parkinson's disease, Alzheimer's disease, Huntington's disease, dementias such as multiinfarct dementia, sclerosis, nerve degeneration associated with diabetes mellitus, disorders affecting the circadian clock or neuro-muscular transmission, and schizophrenia, mood disorders, such as manic depression;
- 3) for treatment of diseases or conditions of the muscles including conditions with impaired function of neuro-muscular connections, such as after organ transplan-

tation, or such as genetic or traumatic atrophic muscle disorders; or for treatment of diseases or conditions of various organs, such as degenerative conditions of the gonads, of the pancreas such as diabetes mellitus type I and II, of the kidney such as nephrosis and of the heart, liver and bowel, and/or

- 5 4) cancer disease, and/or
5) prion diseases.

The invention concerns cancer being any type of solid tumors requiring neoangiogenesis.

10

The invention concerns prion diseases selected from the group consisting of scrapie, Creutzfeldt-Jakob disease. It has been shown that FGFRs plays a distinct role in prion diseases (Castelnau et al. (1994) Exp Neurobiol. 130:407-10; Ye and Carp (2002) J Mol Neurosci. 18:179-88).

15

In another embodiment a compound of the invention is for the manufacture of a medicament for

- 1) promotion of wound-healing, and/or
2) prevention of cell death of heart muscle cells, such as after acute myocardial
20 infarction, or after angiogenesis, and/or
3) revascularisation.

L1 has been broadly studied as a neurite outgrowth stimulator. It has also been regarded important for neural cell precursor proliferation, differentiation and transmitter
25 phenotype subtype generation (Dihne et al. (2003) J Neurosci 23:6638-6650), and it is known to play a role in synaptic plasticity (Saghatelian et al. (2004) Mol Cell Neurosci 26:191-203). FGFRs and their ligands play important roles in CNS, in particular they are involved in the processes associated with memory and learning (Reuss and von Bohlen und Halbach (2003) Cell Tissue Res. 313:139-57). Thus, in still another
30 embodiment a compound of the invention may be used for stimulation of the ability to learn and/or of the short and/or long-term memory. This embodiment is one of the preferred embodiments of the invention.

In yet another embodiments a compound of the invention is for the manufacture of a
35 medicament for the

- 1) prevention of cell death due to ischemia;

2) prevention of body damages due to alcohol consumption;

5 In particular, the invention concerns normal, degenerated or damaged L1 presenting cells. Diseases such as X linked hydrocephalus and MASA syndrome corpus callosum hypoplasia, mental retardation, adducted thumbs, spasticity and hydrocephalus (CRASH) syndrome are of a particular interest of the present invention.

10 The medicament of the invention comprises an effective amount of one or more compounds as defined above, or a pharmaceutical composition comprising one or more compounds and pharmaceutically acceptable additives.

Thus, the invention in another aspect also concerns a pharmaceutical composition comprising at least one compound of the invention.

15 A further aspect of the invention is a process of producing a pharmaceutical composition, comprising mixing an effective amount of one or more of the compounds of the invention, or a pharmaceutical composition according to the invention with one or more pharmaceutically acceptable additives or carriers. In one embodiment the
20 compounds are used in combination with a prosthetic device, wherein the device is a prosthetic nerve guide. Thus, in a further aspect, the present invention relates to a prosthetic nerve guide, characterised in that it comprises one or more of the compounds or the pharmaceutical composition as defined above. Nerve guides are known in the art.

25 The invention relates to use of a medicament and/or pharmaceutical composition comprising the compound of invention for the treatment or prophylaxis of any of the diseases and conditions mentioned below.

30 Such medicament and/or pharmaceutical composition may suitably be formulated for oral, percutaneous, intramuscular, intravenous, intracranial, intrathecal, intracerebroventricular, intranasal or pulmonal administration.

35 Strategies in formulation development of medicaments and compositions based on the compounds of the present invention generally correspond to formulation strate-

gies for any other protein-based drug product. Potential problems and the guidance required to overcome these problems are dealt with in several textbooks, e.g. "Therapeutic Peptides and Protein Formulation. Processing and Delivery Systems", Ed. A.K. Banga, Technomic Publishing AG, Basel, 1995.

5

Injectables are usually prepared either as liquid solutions or suspensions, solid forms suitable for solution in, or suspension in, liquid prior to injection. The preparation may also be emulsified. The active ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like, and combinations thereof. In addition, if desired, the preparation may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH-buffering agents, or which enhance the effectiveness or transportation of the preparation.

15

Formulations of the compounds of the invention can be prepared by techniques known to the person skilled in the art. The formulations may contain pharmaceutically acceptable carriers and excipients including microspheres, liposomes, microcapsules, nanoparticles or the like.

20

The preparation may suitably be administered by injection, optionally at the site, where the active ingredient is to exert its effect. Additional formulations which are suitable for other modes of administration include suppositories, nasal, pulmonal and, in some cases, oral formulations. For suppositories, traditional binders and carriers include polyalkylene glycols or triglycerides. Such suppositories may be formed from mixtures containing the active ingredient(s) in the range of from 0.5% to 10%, preferably 1-2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and generally contain 10-95% of the active ingredient(s), preferably 25-70%.

25

30

35

Other formulations are such suitable for nasal and pulmonal administration, e.g. inhalators and aerosols.

The active compound may be formulated as neutral or salt forms. Pharmaceutically acceptable salts include acid addition salts (formed with the free amino groups of the peptide compound) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic acid, oxalic acid, tartaric acid, mandelic acid, and the like. Salts formed with the free carboxyl group may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The preparations are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective. The quantity to be administered depends on the subject to be treated, including, e.g. the weight and age of the subject, the disease to be treated and the stage of disease. Suitable dosage ranges are per kilo body weight normally of the order of several hundred μg active ingredient per administration with a preferred range of from about 0.1 μg to 5000 μg per kilo body weight. Using monomeric forms of the compounds, the suitable dosages are often in the range of from 0.1 μg to 5000 μg per kilo body weight, such as in the range of from about 0.1 μg to 3000 μg per kilo body weight, and especially in the range of from about 0.1 μg to 1000 μg per kilo body weight. Using multimeric forms of the compounds, the suitable dosages are often in the range of from 0.1 μg to 1000 μg per kilo body weight, such as in the range of from about 0.1 μg to 750 μg per kilo body weight, and especially in the range of from about 0.1 μg to 500 μg per kilo body weight such as in the range of from about 0.1 μg to 250 μg per kilo body weight. In particular, when administering nasally smaller dosages are used than when administering by other routes. Administration may be performed once or may be followed by subsequent administrations. The dosage will also depend on the route of administration and will vary with the age and weight of the subject to be treated. A preferred dosage of multimeric forms would be in the interval 1 mg to 70 mg per 70 kg body weight.

For some indications a localised or substantially localised application is preferred.

For other indications, intranasal application is preferred.

Some of the compounds of the present invention are sufficiently active, but for some of the others, the effect will be enhanced if the preparation further comprises pharmaceutically acceptable additives and/or carriers. Such additives and carriers will be known in the art. In some cases, it will be advantageous to include a compound,
5 which promotes delivery of the active substance to its target.

In many instances, it will be necessary to administrate the formulation multiple times. Administration may be a continuous infusion, such as intraventricular infusion or administration in more doses such as more times a day, daily, more times a
10 week, weekly, etc. It is preferred that administration of the medicament is initiated before or shortly after the individual has been subjected to the factor(s) that may lead to cell death. Preferably the medicament is administered within 8 hours from the factor onset, such as within 5 hours from the factor onset. Many of the compounds exhibit a long term effect whereby administration of the compounds may be
15 conducted with long intervals, such as 1 week or 2 weeks.

In connection with the use in nerve guides, the administration may be continuous or in small portions based upon controlled release of the active compound(s). Furthermore, precursors may be used to control the rate of release and/or site of release.
20 Other kinds of implants and well as oral administration may similarly be based upon controlled release and/or the use of precursors.

Treatment

25 Treatment by the use of the compounds/compositions according to the invention is in one embodiment useful for inducing differentiation, modulating proliferation, stimulate regeneration, neuronal plasticity and survival of cells, for example cells being implanted or transplanted. This is particularly useful when using compounds having a long term effect.

30 In further embodiment the treatment may be for stimulation of survival of cells which are at risk of dying due to a variety of factors, such as traumas and injuries, acute diseases, chronic diseases and/or disorders, in particular degenerative diseases normally leading to cell death, other external factors, such as medical and/or surgical
35 treatments and/or diagnostic methods that may cause formation of free radicals

or otherwise have cytotoxic effects, such as X-rays and chemotherapy. In relation to chemotherapy the FGFR binding compounds according to the invention are useful in cancer treatment.

5 Thus, the treatment comprises treatment and/or prophylaxis of cell death in relation to diseases or conditions of the central and peripheral nervous system, such as postoperative nerve damage, traumatic nerve damage, e.g. resulting from spinal cord injury, impaired myelination of nerve fibers, postischaemic damage, e.g. resulting from a stroke, multiinfarct dementia, multiple sclerosis, nerve degeneration associated with diabetes mellitus, neuro-muscular degeneration, schizophrenia, Alzheimer's disease, Parkinson's disease, or Huntington's disease.

Also, in relation to diseases or conditions of the muscles including conditions with impaired function of neuro-muscular connections, such as genetic or traumatic atrophic muscle disorders; or for the treatment of diseases or conditions of various organs, such as degenerative conditions of the gonads, of the pancreas, such as diabetes mellitus type I and II, of the kidney, such as nephrosis the compounds according to the invention may be used for inducing differentiation, modulating proliferation, stimulate regeneration, neuronal plasticity and survival , i.e. stimulating survival.

20 Furthermore, the compound and/or pharmaceutical composition may be for preventing cell death of heart muscle cells, such as after acute myocardial infarction, in order to induce angiogenesis. Furthermore, in one embodiment the compound and/or pharmaceutical composition is for the stimulation of the survival of heart muscle cells, such as survival after acute myocardial infarction. In another aspect the compound and/or pharmaceutical composition is for revascularisation, such as after injuries.

30 It is also within the scope of the invention a use of the compound and/or pharmaceutical composition for the promotion of wound-healing. The present compounds are capable of stimulating angiogenesis and thereby they can promote the wound healing process.

The invention further discloses a use of the compound and/or pharmaceutical composition in the treatment of cancer. Regulation of activation of FGFR is important for tumor angiogenesis, proliferation and spreading.

- 5 In yet a further embodiment a use of the compound and/or pharmaceutical composition is for the stimulation of the ability to learn and/or of the short and/or long term memory, as FGFR activity is important for differentiation of neural cells.

- 10 In still another embodiment a compound and/or pharmaceutical composition of the invention is for the treatment of body damages due to alcohol consumption. Developmental malformations of foetuses, long-term neurobehavioral alterations, alcoholic liver disease are particularly concerned.

- 15 Therapeutic treatment of prion diseases including using a compound and/or pharmaceutical composition is still another embodiment of the invention.

- In particular the compound and/or pharmaceutical composition of the invention may be used in the treatment of clinical conditions, such as Neoplasms such as malignant neoplasms, benign neoplasms, carcinoma in situ and neoplasms of uncertain
20 behavior, cancer in breast, thyroidal, pancreas, brain, lung, kidney, prostate, liver, heart, skin, blood organ, muscles (sarcoma), cancers with dysfunction and/or over- or under-expression of specific receptors and/or expression of mutated receptors or associated with soluble receptors, such as but not limited to Erb-receptors and FGF-receptors, diseases of endocrine glands, such as diabetes mellitus I and II, pituitary
25 gland tumor, psychoses, such as senile and presenile organic psychotic conditions, alcoholic psychoses, drug psychoses, transient organic psychotic conditions, Alzheimer's disease, cerebral lipidoses, epilepsy, general paresis [syphilis], hepatolenticular degeneration, Huntington's chorea, Jakob-Creutzfeldt disease, multiple sclerosis, Pick's disease of the brain, polyarthritis nodosa, syphilis, Schizophrenic disorders, affective psychoses, neurotic disorders, personality disorders, including character neurosis, nonpsychotic personality disorder associated with organic brain syndromes, paranoid personality disorder, fanatic personality, paranoid personality
30 (disorder), paranoid traits, sexual deviations and disorders or dysfunctions (including reduced sexual drive for what ever reason), mental retardation, disease in the
35 nervous system and sense organs, such as affecting sight, hearing smell, feeling, tast-

ing, cognitive anomalies after disease, injury (e.g. after trauma, surgical procedure, and violence), inflammatory disease of the central nervous system, such as meningitis, encephalitis, Cerebral degenerations such as Alzheimer's disease, Pick's disease, senile degeneration of brain, senility NOS, communicating hydrocephalus, obstructive hydrocephalus, Parkinson's disease including other extra pyramidal disease and abnormal movement disorders, spinocerebellar disease, cerebellar ataxia, Marie's Sanger-Brown, Dyssynergia cerebellaris myoclonica, primary cerebellar degeneration, such as spinal muscular atrophy, familial, juvenile, adult spinal muscular atrophy, motor neuron disease, amyotrophic lateral sclerosis, motor neuron disease, progressive bulbar palsy, pseudobulbar palsy, primary lateral sclerosis, other anterior horn cell diseases, anterior horn cell disease, unspecified, other diseases of spinal cord, syringomyelia and syringobulbia, vascular myelopathies, acute infarction of spinal cord (embolic) (nonembolic), arterial thrombosis of spinal cord, edema of spinal cord, hematomyelia, subacute necrotic myelopathy, subacute combined degeneration of spinal cord in diseases classified elsewhere, myelopathy, drug-induced, radiation-induced myelitis, disorders of the autonomic nervous system, disorders of peripheral autonomic, sympathetic, parasympathetic, or vegetative system, familial dysautonomia [Riley-Day syndrome], idiopathic peripheral autonomic neuropathy, carotid sinus syncope or syndrome, cervical sympathetic dystrophy or paralysis. peripheral autonomic neuropathy in disorders classified elsewhere, amyloidosis, diseases of the peripheral nerve system, brachial plexus lesions, cervical rib syndrome, costoclavicular syndrome, scalenus anticus syndrome, thoracic outlet syndrome, brachial neuritis or radiculitis NOS, including in newborn. Inflammatory and toxic neuropathy, including acute infective polyneuritis, Guillain-Barre syndrome, Postinfectious polyneuritis, polyneuropathy in collagen vascular disease, disorders of the globe including disorders affecting multiple structures of eye, such as purulent endophthalmitis, diseases of the ear and mastoid process, chronic rheumatic heart disease, ischaemic heart disease, arrhythmia, diseases in the pulmonary system, respiratory system, sensoring e.g. oxygene, asthma, abnormality of organs and soft tissues in newborn, including in the nerve system, complications of the administration of anesthetic or other sedation in labor and delivery, diseases in the skin including infection, insufficient circulation problem, burn injury and other mechanic and/or physical injuries. Injuries, including after surgery, crushing injury, burns. Injuries to nerves and spinal cord, including division of nerve, lesion in continuity (with or without open wound), traumatic neuroma (with or without open

wound), traumatic transient paralysis (with or without open wound), accidental puncture or laceration during medical procedure, injury to optic nerve and pathways, optic nerve injury, second cranial nerve, injury to optic chiasm, injury to optic pathways, injury to visual cortex, unspecified blindness, injury to other cranial nerve(s), injury to other and unspecified nerves. Poisoning by drugs, medicinal and biological substances, genetic or traumatic atrophic muscle disorders; or for the treatment of diseases or conditions of various organs, such as degenerative conditions of the gonads, of the pancreas, such as diabetes mellitus type I and II, of the kidney, such as nephrosis. Scrapie, Creutzfeldt-Jakob disease, Gerstmann-Straussler-Sheinker (GSS) disease; X linked hydrocephalus and MASA syndrome corpus callosum hypoplasia, mental retardation, adducted thumbs, spasticity and hydrocephalus (CRASH) syndrome, pain syndrome, encephalitis, drug/alcohol abuse, anxiety, postoperative nerve damage, peri-operative ischemia, inflammatory disorders with tissue damage, either by affecting the infections agent or protecting the tissue, HIV, hepatitis, and following symptoms, autoimmune disorders, such as rheumatoid arthritis, SLE, ALS, and MS. Anti-inflammatory effects, asthma and other allergic reactions, acute myocardial infarction, and other related disorders or sequel from AMI, metabolic disorders, such as obesity lipid disorders (e.g. hyper cholestrolamia, artherosclerosis, disorders of amino-acid transport and metabolism, disorders of purine and pyrimidine metabolism and gout, bone disorders, such as fracture, osteoporosis, osteo arthritis (OA), Atrophic dermatitis, psoriasis, infection caused disorders, stem cell protection or maturation in vivo or in vitro.

According to the invention the treatment and/or prevention of the above conditions and symptoms comprises a step of administering an effective amount of a compound and/or pharmaceutical composition to an individual in need.

Examples

1. L1 fragments of the invention

30	SEQ ID NO :1	APEKWFSLGKV	F1CDL peptide
	SEQ ID NO: 2	DWNAPQIQYRVQWR	F2BCL peptide
	SEQ ID NO: 3	DLAQVKGHLRGYN	F3ABL peptide
	SEQ ID NO: 4	RHVHKSHMVVPAN	F3CDL peptide
	SEQ ID NO: 5	RFHILFKALPEGKVSPD	F5BCL peptide

SEQ ID NO: 6 LHHLAVKTNGTG F5FGL peptide

2. Experimental Procedures

2. 1. Production of recombinant proteins

5 The L1 F3 modules I-V were produced by RT-PCR using rat brain total RNA. The FGFR1 Ig modules II-III were produced using mouse FGFR1 (IIIC isoform) cDNA (kindly provided by Dr. Patrick Doherty, King's College, London). The L1 F3 modules consist of RSPWPG, amino acids 608-1109 of L1 (swissprot Q05695) and HHHHHH. The FGFR1 Ig module II consist of RSHHHHHH and amino acids 253-10 365 of FGFR1 (swissprot p16092). The FGFR1 combined Ig modules II-III consist of RSHHHHHH and amino acids 141-365 of FGFR1 (swissprot p16092). The FGFR1 Ig modules II-III and L1 F3 modules I-V were expressed in Drosophila S2 cells (Invitrogen, USA) according to the manufacturer's instructions. All the proteins were purified by affinity chromatography using Ni²⁺-NTA resin (Qiagen, USA), ion exchange chromatography, and gel filtration (all chromatography columns used for purification were bought from Amersham Biosciences, Sweden). The IgII module of FGFR1 was expressed in Pichia Pastoris KM71 cells expression system (Invitrogen, USA) according to the manufacturer's instructions. The protein was purified by affinity chromatography using Ni²⁺-NTA resin (Qiagen, USA), ion exchange chromatography and gel filtration (all chromatography columns used for purification were bought from Amersham Biosciences, Sweden). The combined NCAM Ig modules I and II (RV and amino acids 20-208 of rat NCAM, swissprot p13596) were produced as described (Jensen et. al., 1999).

25 2.2 SPR Analysis

Binding analysis was performed using a BIAcoreX instrument (Biosensor AB, Sweden) at 25 °C using 10 mM sodium phosphate pH 7.4, 150 mM NaCl as running buffer. The flow-rate was 5 l/min. Data were analysed by non-linear curve-fitting using the manufacturer's software. The FGFR1 Ig modules 2-3 were immobilized on the sensor chip CM5 using the amine coupling kit (Biosensor AB) as follows: 1) the two halves of the chip (designated Fc1 and Fc2) were activated by 20 l activation solution; 2) the protein was immobilized on Fc1 using 12 l 20 g/ml protein in 10 mM sodium phosphate buffer pH 6.0; 3) Fc1 and Fc2 were blocked by 35 l blocking solu-

tion. Binding of various compounds to the immobilized FGFR1 modules was studied as follows: A compound was injected simultaneously into Fc1 (with the immobilized FGFR1 modules) and Fc2 (with nothing immobilized). The curve representing un-specific binding of the compound to the surface of Fc2 was subtracted from the curve representing binding of the protein to the immobilized FGFR1 modules and the surface of Fc1. The resulting curve was used for analysis. For ATP competition experiments various compounds were preincubated for 10 min with ATP at a specified concentration. The affinity between ATP and the L1 F3 modules was estimated from the following procedure: the initial binding rates of 14 μ M F3 modules, V_0 , and of 14 μ M F3 modules preincubated with ATP at a specified concentration, V_{ATP} , were determined. V_{ATP} can be calculated from the equation:

$$V_0 = k_1 F + \frac{1}{2} \left((F + a + K_D) - \sqrt{(F + a + K_D)^2 - 4Fa} \right) (k_2 - k_1),$$
 where k_2 and k_1 are proportionality constants between the initial binding rate and concentration at the initial moment for the ATP-F3 complex and F3 modules, respectively; F is the concentration of the F3 modules; a is the concentration of ATP; K_D is the equilibrium dissociation constant. V_{ATP} was plotted against the ATP concentration, and K_D was calculated by non-linear fitting of the theoretical curve to the experimental data.

2.3 Assays for determination of FGFR1 phosphorylation

TREX-293 cells (Invitrogen) were stably transfected with human FGFR1 (kindly supplied by Dr. Lena Claesson-Welsh, Uppsala, Sweden) with a C-terminal StrepII-tag (IBA biotech, Germany), using the Flp-In system (Invitrogen). For the study of phosphorylation: $\sim 1 \times 10^6$ TREX/FGFR1 cells were starved overnight before stimulation for 15 min with the specified compounds. Cells were lysed in PBS with 1% NP-40, Complete protease inhibitors (Roche, Switzerland) and the phosphatase inhibitor cocktail set II (Calbiochem, USA). Cleared cell lysates were analysed for total protein content using Pierce BCA assay (Pierce, USA) and equal amounts of protein were incubated with 20 μ l agarose-coupled anti-phosphotyrosine antibodies (4G10-AC, Upstate Biotechnologies, USA) for 3 hrs at 4°C. The bound protein was washed in lysis buffer (x1) and PBS (x2) before elution in 150 mM phenyl phosphate (Sigma, Germany). Protein in the elution fraction was precipitated with 12% trichloroacetic acid, washed in cold acetone and dissolved in SDS-PAGE sample buffer. Immunoblotting was performed using antibodies against the StrepII-tag (IBA Biotech).

2. 4 Cerebellar granule neuron culture and immunostaining

Dissociated neurons from rat cerebellum (postnatal day 7-8) were grown on 8-well Lab-Tek chamber slides (Nunc, Germany) (10^4 cells per each well) for 24 h at 37°C, 5% CO₂ in Neurobasal medium (Gibco) containing 20 mM Hepes, 100 U/ml penicillin/ streptomycin, 1% glutamax, 25 mg/L pyruvate, 0.4% BSA (Sigma) supplemented with B27 (Gibco) and the specified compounds. After 24 h, cells were fixed with paraformaldehyde, immunostained with primary antibodies against GAP -43 (1:1000) (Chemicon, USA), secondary Alexa Flour antibodies (1:700) (Molecular Probes, Netherlands) and the length of neurites was measured by a stereological method as described (Rønn et al., 2000).

3. Results

To study the possible interaction between the L1 F3 modules and FGFR1, we used the following recombinant proteins: the combined NCAM Ig modules I-II, the combined L1 F3 modules I-V, FGFR Ig module II, and the combined FGFR1 Ig modules II-III. The NCAM and FGFR Ig modules were properly folded as judged by one-dimensional NMR analysis and the L1 F3 modules were properly folded since the extracellular part of L1 (all the Ig and F3 modules together) expressed by the same procedure could be crystallized (Kulahin et al., 2004).

3.1 Demonstration of a direct interaction between L1 and FGFR

To test whether the L1 F3 modules could bind to FGFR1, we used SPR analysis. From Fig. 1, it appears that the recombinant protein consisting of the L1 F3 modules I-V bound to the immobilized FGFR1 Ig modules II-III, whereas a control protein consisting of the NCAM Ig modules I-II did not bind to the FGFR1 fragment (Fig. 1). The equilibrium dissociation constant (K_d) and the coefficients of association and dissociation were: $3.246 \pm 0.452 \mu\text{M}$, $321 \pm 65 \text{ M}^{-1}\text{s}^{-1}$ and $1.09 \pm 0.003 \times 10^{-3} \text{ s}^{-1}$ (mean $\pm$ standard deviation), respectively. Thus, these data indicate that the L1 F3 modules bind directly to FGFR1.

3.2 Binding of L1 to FGFR1 can be enhanced by ATP, GTP and AMP-PCP

It has been shown that the binding between NCAM and FGFR1 can be inhibited by ATP (Kiselyov et al., 2003). It was therefore of interest to see whether ATP had the same effect for the L1-FGFR1 binding. To test this we used SPR analysis. As can be seen from Fig. 2A and 3A, adding ATP to 14 μM L1 F3 modules I-V enhanced

the binding of the F3 modules to FGFR1 Ig modules II-III. In order to test whether this effect was specific for ATP, AMP-PCP (a non-hydrolysable analogue of ATP) (Fig. 2B, 3A), AMP (Fig. 2C, 3A), and GTP (Fig. 2D, 3A), were tested in the same setup. GTP and AMP-PCP also bound to the L1 F3 modules increasing the affinity of the interaction between the modules and FGFR1, whereas AMP demonstrated insignificant inhibition of the binding (Fig. 2C, 3A). These results indicated that ATP, GTP and AMP-PCP binding to the L1 F3 modules may change the conformation of the protein thereby increasing the affinity between L1 and FGFR1. The K_d of the interaction between 14 μM L1 F3 modules and FGFR1 in the presence of 2mM ATP (GTP or AMP-PCP) decreased from $3.246 \pm 0.452 \mu\text{M}$ to $1.246 \pm 0.129 \mu\text{M}$ ($0.944 \pm 0.041 \mu\text{M}$ or $0.861 \pm 0.032 \mu\text{M}$, respectively) (Fig. 3A). Fitting experimental data using a model, where ATP binds to the L1F3 modules and changes the initial rate of the binding, we estimated the equilibrium dissociation constants (K_d) of the interaction between the L1 F3 modules and ATP, which appeared to be $0.388 \pm 0.105 \text{ mM}$ (Fig. 3B). These experiments indicate that only triphosphate nucleotides can enhance the L1-FGFR1 interaction.

3.3 FGFR1 is activated by the L1 F3 modules

Since the L1 F3 modules bind to FGFR1, they may be expected to induce FGFR1 activation in living cells. To test this, we used a method for detecting FGFR1 phosphorylation previously described by Kiselyov et al. (2003). TREX-293 cells, stably transfected with FGFR1 containing a C-terminal StrepII-tag, were stimulated with the F3 modules of L1 at different concentrations. After stimulation, FGFR1 was immunopurified using a fixed amount of anti-phosphotyrosine antibodies and then analyzed by immunoblotting using antibodies against the StrepII-tag. As appears from Fig. 4 that L1 F3 modules substantially increased FGFR1 phosphorylation compared to the non-stimulated cells and cells treated with the combined NCAM Ig I-II modules which did not bind to FGFR1 by SPR analysis (see Fig. 1). The highest phosphorylation level was achieved at a concentration of the L1 F3 modules of 10 μM . This indicates that binding of the L1 F3 modules to FGFR1 results in the activation of the receptor.

FGFR1 activation by the L1 F3 modules stimulates neurite outgrowth and this stimulatory effect can be modulated by ATP

Since the L1 F3 modules activate FGFR1, we suggested that they were capable of mimicking a characteristic function of L1: neuronal differentiation as reflected by neurite outgrowth. To test this assumption, dissociated cerebellar neurons were seeded on plastic and allowed to grow for 24 h in the presence of the below described compounds. As can be seen from Fig. 5A, at a concentration of 4 μ M the L1 F3 modules substantially increased the length of neurites compared to non-stimulated neurons. The effect was quantified in a dose-response study demonstrating that the F3 modules induced neurite outgrowth with a bell-shaped curve typical of growth factor induced neurite outgrowth (Hatten et al., 1988). The stimulatory effect of the F3 modules could be completely abrogated by an inhibitor of FGFR1, SU5402 (Fig. 5B), further supporting the notion that the modules interact with FGFR1.

Since ATP enhanced the L1-FGFR1 binding, we presumed that ATP might interfere with the FGFR1 activation by the L1 F3 modules, and, consequently, affect the neurite promoting activity of the modules. To test this, neurons were stimulated with the L1 F3 modules in the presence of ATP or AMP-PCP. As can be seen from Fig. 5C and D, both ATP and AMP-PCP substantially reduced the neuritogenic effect induced by the F3 modules when using a concentration of the L1 F3 modules (4 μ M) giving a maximal response in the absence of ATP (Fig. 5A). A complete inhibition of the effect of F3 modules was achieved with AMP-PCP at lower concentrations in comparison to the concentration of ATP, indicating that ATP was a less potent inhibitor than its non-hydrolysable analogue. However, when the concentration of the L1 F3 modules was lowered to 1 μ M the addition of ATP or AMP-PCP increased the effect of the protein on neurite outgrowth (Fig. 5E and F). It indicates that adding ATP that increases the affinity between L1 and FGFR1, led to a higher ratio between bound and unbound FGFR1 to L1 as it happened when the L1 F3 modules were added at a higher concentration (Fig. 5A). Therefore the concentration of the F3 modules should be lower than the most effective concentration to demonstrate the enhancement of neurite outgrowth by adding ATP. These results indicate that the activation of FGFR1 in neurons by the L1 F3 modules induced neuritogenesis and this effect could be modulated by ATP.

3.4 Mapping the binding site between L1 F3 modules and FGFR1

Since all F3 modules of L1 constitute a protein which is longer than FGFR1 it was of interest to localize a part of L1 responsible for the binding to FGFR1. The secondary structure of a fibronectin type 3 module of L1 consists of two beta-sheets containing seven antiparallel beta-strands A, C, E, G and B, D, F, respectively, which fold up to form a beta-sandwich. Recently binding between the F3 modules of NCAM has been demonstrated (Kiselyov et al., 2003) to take place in the region of NCAM corresponding to the loop between the F and G beta-strands of F3II which is close to the N-terminus of F3II and C-terminus of F3I modules of NCAM. Since NCAM and L1 are structurally homologous proteins it was suggested that the binding between L1 and FGFR1 also takes place in loop regions of F3 modules of L1 similar to the model of binding between NCAM and FGFR1. Thus, all thirty loop regions of L1 F3 modules (Fig. 6) were produced as peptides and binding between the peptides and FGFR1 was tested using SPR analysis. From the Figure 7A it is possible to see that peptides corresponding to loop regions between C and D beta-strands of F3I module (F1CDL-peptide, APEKWFSLGKV (SEQ ID NO: 1)), between B and C beta-strands of F3II module (F2BCL-peptide, DWNAPQIQYRVQWR (SEQ ID NO: 2)), between A and B beta-strands of F3III module (F3ABL-peptide, DLAQVKGHLRGYN (SEQ ID NO: 3)), between C and D beta-strands of F3III module (F3CDL-peptide, RHVHKSHMVVPAN (SEQ ID NO: 4)), between B and C beta-strands of F3V module (F5BCL-peptide, AA: RFHILFKALPEGKVSPD (SEQ ID NO: 5)) and between F and G beta-strands of F3V module (F5FGL-peptide, AA: LHHLAVKTNGTG (SEQ ID NO: 6)) bound to Ig modules II-III of FGFR1. Since both IgII and IgIII modules of FGFR1 can be responsible for the binding between L1 and FGFR1 it was of interest to examine binding of the peptides to the single modules of FGFR1. The same setup of SPR experiments was used to demonstrate that four out of six peptides (F1CDL-, F2BCL-, 3CDL-, and 5BCL-peptides) bound to IgII module of FGFR1 (Fig. 7B). These data demonstrate that there might be 6 binding sites in fibronectin modules of L1 responsible for binding between L1 and FGFR1.

3.5 Peptides binding to FGFR1 activate the receptor and stimulate neurite outgrowth.

Since peptides representing some loop regions of the F3 modules bound to FGFR1 it was suggested that they could activate FGFR1. To check whether it is true the same method as for investigation of activation of FGFR1 by F3 modules was used for the peptides. From Figure 8 it appears that F1CDL, F2BCL, F3ABL, F5BCL and

F5FGL peptides increased significantly the level of phosphorylation of the receptor, compared to the control peptide (F1BCL-peptide was used as a negative control since it did not bind to FGFR1 and did not show dose-dependent phosphorylation of the receptor). At the same time F3CDL peptide demonstrated much smaller effect on phosphorylation of the FGFR1.

Since FGFR1 was activated by the peptides we suggested that they could mimic an above described property of the F3 modules of L1 to stimulate neurite outgrowth. The same approach was used to show that F1CDL, F2BCL, F3ABL, F5BCL and F5FGL peptides (the peptides which increased phosphorylation level of the FGFR1) stimulated neurite outgrowth whereas F3CDL and F1BCL peptides (the latter did not bind to the FGFR1) did not lead to neurite outgrowth response compared to control (Fig. 9).

Based on these data we proposed a model for binding between the F3 modules of L1 and FGFR1 (Fig. 10; dimer of FGFR1 was a model based on the crystal structure of FGFR1; Pellegrini et al., 2000). It could be argued whether it is possible that the F3 modules can have such a compact conformation. Therefore possible conformation of the protein was estimated based on gel-filtration chromatography. From Figure 11 it is possible to see that F3 modules of L1 elute from the Superdex 200 pg column in PBS later than when the gel-filtration is performed in PBS plus 1 M NaCl, whereas elution time of BSA in both buffers remained the same. These data indicate that the F3 modules of L1 might have a relatively compact conformation which is in accordance with the above described model of binding between L1 and FGFR1.

4. Discussion

We here present the first direct evidence of an interaction between the neuronal cell adhesion molecule L1 and FGFR1. Moreover, we demonstrate a regulatory role of ATP in this interaction.

Using SPR analysis, we have shown that L1 membrane proximal F3 modules I-V bound to FGFR1 membrane proximal Ig modules II-III with a K_d of $3.246 \pm 0.452 \mu\text{M}$. A close association of FGFR1 and L1 was also demonstrated in living cells by immunoprecipitation.

Stimulation of cells with the L1 F3 modules increased FGFR1 phosphorylation. This raises the question how the modules induce FGFR1 activation. One explanation

may be that FGFR1 has an intrinsic ability to dimerise, although with a very low affinity. This leads to a dynamic equilibrium in which a small fraction of FGFR1 molecules forms transient dimers, resulting in a low background level of FGFR1 phosphorylation. FGFR1 binding of any ligand may change the dimerisation slightly, shifting the equilibrium to either association or dissociation. Thus, we presume that binding of the F3 modules to FGFR1 may shift the equilibrium towards association and, as a result, increase the FGFR1 phosphorylation.

Besides binding to and activating FGFR1, the F3 modules were capable of mimicking a characteristic function of L1: promotion of neurite outgrowth. Stimulation of the cells by the F3 modules could be blocked by an inhibitor of L1-stimulated neurite outgrowth, the FGFR1 inhibitor SU5402, further supporting the notion that the modules interact with FGFR1.

Using SPR analysis, we observed that ATP enhanced the binding of the F3 modules to FGFR1 Ig modules II-III. We also demonstrated that ATP modulated the stimulatory effect of the F3 modules on outgrowth of neurites. This effect depended on the concentration of the L1 F3 modules that was used to stimulate neurite outgrowth. If the concentration of the protein corresponded to the one with the maximal effect on neurite outgrowth induction (Fig. 5) ATP acted inhibitory. On the other hand, if the concentration of the protein was lower, ATP enhanced the induction of neurite outgrowth. This may indicate that when the ratio between bound and unbound FGFR1 to L1 was too high an abrogation of the stimulatory effect of L1 on neurite outgrowth occurred, in accordance with the dose-response study of the effect. Based on these data we therefore suggest that ATP binding to L1 enhances the L1-FGFR1 interaction leading to either stimulation or inhibition of neurite outgrowth depending on the actual concentration of L1.

The experimentally determined value of the affinity for the L1-FGFR1 interaction (K_D of 3 μ M) seems low, but when the affinity of the interaction was increased by addition of ATP, an inhibition of the L1 F3 modules induced neurite outgrowth was observed, meaning that a low affinity of the interaction between FGFR1 and L1 may be an intrinsic property of the cell adhesion molecules L1 and NCAM (the latter has approx. the same affinity to FGFR1; Kiselyov et al., 2003).

Our results elucidate a regulatory role of ATP in L1 mediated neurite extension, which has not been demonstrated previously, although a series of studies have shown that NCAM and other cell adhesion molecules have ecto-ATPase activity and

that ATP seems to inhibit NCAM binding to FGFR1 (Dzhandzhugazyan and Bock, 1997; Kiselyov et al., 2003).

L1 is a very long molecule having 11 modules in the extracellular part. Using synthesized peptides mimicking loop regions of L1 we demonstrated that a possible
5 model for binding between L1 and FGFR1 requires compact conformation of F3 modules. Gel-filtration experiments together with other studies suggesting a horse-shoe shape of the first Ig modules of L1 (Su et al. 1998; Freigang et al. 2000) allow presuming that all 11 modules of L1 have a compact structure.

Homophilic interaction between L1 on a growth cone and L1 expressed by surround-
10 ing cells probably leads to L1 clustering in the growth cone, followed by local increasing the ratio between concentrations of L1 and FGFR1 and, consequently, the activation of the latter, thus providing an environment stimulating axon extension. ATP is an abundant neurotransmitter in the CNS. Thus, when a growth cone reaches its target and a synaptic contact is formed, the release of ATP from the
15 synapse may regulate the coupling between L1 and FGFR1 and, therefore, affect, e. g. inhibit axonal growth in the area of a newly formed synaptic contact, where it is no longer necessary. Conversely, ATP release may trigger L1-FGFR1 interactions and subsequent signaling in areas with a relatively low density of L1 molecules.

Thus, our results provide evidence for a direct interaction between L1 and FGFR1
20 and indicate that ATP is a regulator of L1 induced axonal outgrowth through regulation of the L1-FGFR1 interaction.

References

Doherty P, Williams E, Walsh FS. (1995) A soluble chimeric form of the L1 glycoprotein stimulates neurite outgrowth. *Neuron*. 14(1), 57-66.
25

Dzhandzhugazyan K, Bock E (1997). Demonstration of an extracellular ATP-binding site in NCAM: functional implications of nucleotide binding. *Biochemistry*. 36(49), 15381-95.

30 Felsenfeld DP, Hynes MA, Skoler KM, Furley AJ, Jessell TM. (1994) TAG-1 can mediate homophilic binding, but neurite outgrowth on TAG-1 requires an L1-like molecule and beta 1 integrins. *Neuron*. 12(3), 675-90.

- Freigang J, Proba K, Leder L, Diederichs K, Sonderegger P, Welte W. (2000) The crystal structure of the ligand binding module of axonin-1/TAG-1 suggests a zipper mechanism for neural cell adhesion. *Cell*. 12;101(4), 425-33.
- 5 Grumet M, Edelman GM. (1984) Heterotypic binding between neuronal membrane vesicles and glial cells is mediated by a specific cell adhesion molecule. *J Cell Biol*. 98(5), 1746-56.
- Hatten, M.E., Lynch, M., Rydel, R.E., Sanchez, J., Joseph-Silverstein, J., Moscatelli,
10 D., Rifkin, D.B. (1988). In vitro neurite extension by granule neurons is dependent upon astroglial-derived fibroblast growth factor. *Dev Biol*. 125(2), 280-9.
- Horstkorte R, Schachner M, Magyar JP, Vorherr T, Schmitz B (1993). The fourth immunoglobulin-like domain of NCAM contains a carbohydrate recognition domain
15 for oligomannosidic glycans implicated in association with L1 and neurite outgrowth. *J Cell Biol*. 121(6), 1409-21.
- Jensen PH, Soroka V, Thomsen NK, Ralets I, Berezin V, Bock E, Poulsen FM (1999). Structure and interactions of NCAM modules 1 and 2, basic elements in
20 neural cell adhesion. *Nat Struct Biol*. 6(5), 486-93.
- Kim I, Moon S, Yu K, Kim U, Koh GY. (2001) A novel fibroblast growth factor receptor-5 preferentially expressed in the pancreas(1). *Biochim Biophys Acta*. 19;1518(1-2), 152-6.
- 25 Kuhn TB, Stoeckli ET, Condrau MA, Rathjen FG, Sonderegger P (1991). Neurite outgrowth on immobilized axonin-1 is mediated by a heterophilic interaction with L1(G4). *J Cell Biol*. 115(4), 1113-26.
- 30 Lemmon V, Farr KL, Lagenaur C. *Neuron*. (1989) L1-mediated axon outgrowth occurs via a homophilic binding mechanism. 2(6), 1597-603.
- Margolis RK, Rauch U, Maurel P, Margolis RU (1996). Neurocan and phosphacan: two major nervous tissue-specific chondroitin sulfate proteoglycans. *Perspect Dev
35 Neurobiol*. 3(4), 273-90

- Nybroe O, Bock E (1990). Structure and function of the neural cell adhesion molecules NCAM and L1. *Adv Exp Med Biol.* 265, 185-96.
- 5 Plotnikov AN, Schlessinger J, Hubbard SR, Mohammadi M. (1999) Structural basis for FGF receptor dimerization and activation. *Cell.* 3;98(5), 641-50.
- 10 Powers CJ, McLeskey SW, Wellstein A (2000). Fibroblast growth factors, their receptors and signaling. *Endocr Relat Cancer.* 7(3),165-97.
- Ronn LC, Ralets I, Hartz BP, Bech M, Berezin A, Berezin V, Moller A, Bock E (2000). A simple procedure for quantification of neurite outgrowth based on stereological principles. *J Neurosci Methods.* 100(1-2), 25-32.
- 15 Sammar M, Aigner S, Altevogt P (1997). Heat-stable antigen (mouse CD24) in the brain: dual but distinct interaction with P-selectin and L1. *Biochim Biophys Acta.* 1337(2), 287-94.
- 20 Su XD, Gastinel LN, Vaughn DE, Faye I, Poon P, Bjorkman PJ. (1998) Crystal structure of hemolin: a horseshoe shape with implications for homophilic adhesion. *Science* 14;281(5379), 991-5.
- 25 Vladislav V. Kiselyov, Galina Skladchikova, Anders M. Hinsby, Peter Holme Jensen, Nikolaj Kulahin, Vladislav Soroka, Nina Pedersen, Victor Tsetlin, Flemming M. Poulsen, Vladimir Berezin, and Elisabeth Bock (2003). *Structural Basis for a Direct Interaction between FGFR1 and NCAM and Evidence for a Regulatory Role of ATP.* *Structure* 11(6), 691-701.
- 30 Williams EJ, Furness J, Walsh FS, Doherty P (1994). Activation of the FGF receptor underlies neurite outgrowth stimulated by L1, N-CAM, and N-cadherin. *Neuron.* 13(3), 83-94.

Claims

1. A compound capable of interacting with Fibroblast Growth Factor Receptor (FGFR) comprising an amino acid sequence of 5 to 105 amino acid residues which comprises an amino acid motif of the formula

5 $x^-(x)_n-x^p-(x)_n-x^-$,

wherein

x^- is a basic amino acid residue,

x^p is a hydrophobic amino acid residue and

$(x)_n$ is a sequence of any amino acid residues, wherein n is an integer from 0 to

10 3.
2. The compound according to claim 1, wherein the amino acid motif is a FGFR binding motif.
- 15 3. The compound according to claims 1 or 2, wherein the amino acid motif is any one of the following amino acid motifs:

KxxxLxK, RxxxLxK, KxLxxxK, HxLxxK, RxxWR, KxxLR, RxVxxH,

wherein K, L, R, H, W and V are amino acid residues K, L, R, H, W and V correspondingly, and x is any amino acid residue.

20
4. The compound according to claim 3, wherein at least one x is a basic amino acid residue, hydrophilic amino acid residue or G.
5. The compound according to claim 4, wherein the basic amino acid residue is H.
- 25 6. The compound according to claim 4, wherein the hydrophilic amino acid residue is Q or S.
7. The compound according to claims 1-6, wherein the compound comprises

30

 - i) the first Fibronectin type-III (Fn3,1) module of neural cell adhesion molecule L1 or a fragment thereof, and/or
 - ii) the second Fibronectin type-III (Fn3,2) module of neural cell adhesion molecule L1 or a fragment thereof, and/or
 - 35 iii) the third Fibronectin type-III (Fn3,3) module of neural cell adhesion

- molecule L1 or a fragment thereof, and/or
- iv) the fourth Fibronectin type-III (Fn3,4) module of neural cell adhesion molecule L1 or a fragment thereof, and/or
- v) the fifth Fibronectin type-III (Fn3,5) module of neural cell adhesion molecule L1 or a fragment thereof.
- 5
8. The compound of any of the preceding claims, wherein the compound is a fragment of L1 Fn3,1 and/or L1 Fn3,2, and/or L1 Fn3,3, and/or L1 Fn3,4, and/or L1 Fn3,5 which comprises an amino acid sequence of 5-7 amino acid residues comprising the FGFR binding motif according to claims 1-6.
- 10
9. The compound according to claim 8, wherein the fragment comprises 3 to 90 amino acid residues.
- 15
10. The compound of any of the preceding claims, wherein the compound comprises at least one of the amino acid sequences selected from APEKWFSLGKV (SEQ ID NO: 1), DWNAPQIQYRYQWR (SEQ ID NO: 2), DLAQVKGHLRGYN (SEQ ID NO: 3),
- 20
- RHVHSHMVPAN (SEQ ID NO: 4), RFHILFKALPEGKVSPD (SEQ ID NO: 5) or LHHLAVKTNGTG (SEQ ID NO: 6), or a fragment, a variant thereof.
- 25
11. The compound according to claim 10, wherein the compound consists of at least one of the amino acid sequences selected from the amino acid sequences of SEQ ID NOs: 1-6 or a fragment or variant thereof.
- 30
12. The compound according to claim 11, wherein the compound consists of the amino acid sequence of SEQ ID NO: 1, or a fragment or variant thereof.
13. The compound according to claim 11, wherein the compound consists of the amino acid sequence of SEQ ID NO: 2, or a fragment or variant thereof.
- 35
14. The compound according to claim 11 wherein the compound consists of the

amino acid equence of SEQ ID NO: 3, or a fragment or variant thereof.

15. The compound according to claim 11, wherein the compound consists of the amino acid equence of SEQ ID NO: 4, or a fragment or variant thereof.
- 5 16. The compound according to claim 11, wherein the compound consists of the amino acid equence of SEQ ID NO: 5, or a fragment or variant thereof.
- 10 17. The compound according to claim 11, wherein the compound consists of the amino acid equence of SEQ ID NO: 6, or a fragment or variant thereof.
18. The compound according to claim 10, wherein the compound comprises two or more amino acid sequences selected from the sequences of SEQ ID NOs:1-6, or two or more fragments or variants of said sequences.
- 15 19. The compound according to claim 18, wherein the compound comprises two identical copies of an amino acid sequence selected from of the sequences of SEQ ID NOs:1-6, or two identical fragments or variants of the selected sequence, wherein said amino acid sequences, fragments or variants.
- 20 20. The compound according to claim 18, wherein the compound comprises four identical copies of an amino acid sequence selected from the sequences of SEQ ID NOs:1-6, or four identical fragments or variants of the selected sequence, wherein said amino acid sequences, fragments or variants.
- 25 21. The compound according to claim 10, wherein the compound comprises two or more different amino acid sequences, wherein at least one of the two amino acid sequences is a sequence selected from the sequences of SEQ ID NOs: 1-6, or fragments or variants thereof.
- 30 22. The compound according to claim 18, wherein the compound comprises two different amino acid sequences, wherein both amino acid sequences are selected from the sequences of SEQ ID NO: 1-6, or fragments or variants thereof.
- 35 23. The compound according to any of the claims 18-22, wherein the amino acid

sequences are connected to each other through a linker grouping.

- 5 24. The compound according to any of the claims 10-22, wherein the variant is an amino acid sequence which has at least 60% identity with a sequence selected from the sequences of SEQ ID NOs: 1-6 and is capable of binding to FGFR.
25. The compound according to any of the claims 10-22, wherein the fragment comprises the FGFR binding motif according to claims 1-6.
- 10 26. The compound according to any of the preceding claims, wherein FGFR is selected from Fibroblast Growth Factor Receptor 1 (FGFR1), Fibroblast Growth Factor Receptor 2 (FGFR2), Fibroblast Growth Factor Receptor 3 (FGFR3), Fibroblast Growth Factor Receptor 4 (FGFR4) or Fibroblast Growth Factor Receptor 5 (FGFR5).
- 15 27. The compound according to any of the preceding claims, wherein the compound is capable of activating FGFR.
- 20 28. The compound according to claim 27, wherein the compound is capable of stimulating FGFR signalling.
29. The compound according to claim 28, wherein FGFR1 signalling is stimulated.
- 25 30. The compound according to any of the preceding claims, wherein the interaction of the compound with FGFR can be modulated by a nucleotide.
- 30 31. The compound according to claim 30, wherein the interaction is enhanced.
32. The compound according to claim 31, wherein the nucleotide is a nucleotide triphosphate.
33. The compound according to claim 30, wherein the interaction is weakened.
- 35 34. The compound according to claim 33, wherein the nucleotide is a nucleotide mono-phosphate.

35. The compound according to any of the preceding claims 1-29, wherein the compound is capable of stimulating neurite outgrowth.
- 5 36. The compound according to any of the preceding claims 1-29, wherein the compound is capable of stimulating cell survival.
37. The compound according to any of the preceding claims 1-29, wherein the compound is capable of stimulating synaptic plasticity.
- 10 38. The compound according to any of the preceding claims 1-29, wherein the compound is capable of stimulating differentiation of stem cells.
39. The compound according to any of the claims 1-29, wherein the compound is capable of stimulating learning and/or memory.
- 15 40. A pharmaceutical composition comprising at least one compound as defined in any of the claims 1-39.
- 20 41. The pharmaceutical composition according to claim 40, said composition is formulated for oral, percutaneous, intramuscular, intravenous, intracranial, intrathecal, intracerebroventricular, intranasal or pulmonal administration.
42. The pharmaceutical composition according to claim 41, wherein the administration is continuous.
- 25 43. A process of producing a pharmaceutical composition, comprising mixing an effective amount of one or more of the compounds according to the claims 1-39, or a pharmaceutical composition according to any of the claims 40-42 with one or more pharmaceutically acceptable additives or carriers.
- 30 44. The process according to claim 43, wherein the compound as defined in the claims 1-39 are used in combination with a prosthetic device.
- 35 45. The process according to claim 44, wherein the device is a prosthetic nerve

guide.

- 5 46. The process according to claim 45, wherein the prosthetic nerve guide is characterised in that it comprises at least one compound as defined in any of the claims 1-39, or a pharmaceutical composition as defined in any of the claims 40-43.
- 10 47. Use of a compound as defined in any of the claims 1 to 39 for the manufacture of a medicament for treatment a disease or condition wherein stimulating neurite outgrowth, cell survival, synaptic plasticity, stem cell differentiation and/or learning and memory is beneficial for recovery from said disease of condition.
- 15 48. The use according to claim 47, wherein the medicament is for the treatment of diseases or conditions of the central and peripheral nervous system, postoperative nerve damage, traumatic nerve damage, impaired myelination of nerve fibers, postischaemic damage, multiinfarct dementia, multiple sclerosis, nerve degeneration associated with diabetes mellitus, neuro-muscular degeneration, schizophrenia, mood disorders, manic depressive disorders, Alzheimer's disease, Parkinson's disease, or Huntington's disease.
- 20 49. The use according to claim 47, wherein the medicament is for the treatment of diseases or conditions of the muscles including conditions with impaired function of neuro-muscular connections, or for the treatment of diseases or degenerative conditions of the gonads, pancreas or kidney.
- 25 50. The use according to claim 47, wherein the medicament is for preventing cell death of heart muscle cells.
- 30 51. The use according to claim 47, wherein the medicament is for revascularisation.
52. The use according to claim 47, wherein the medicament is for the promotion of wound healing.
- 35 53. The use according to claim 47, wherein the compound and/or pharmaceutical composition is capable of inhibiting angiogenesis.

54. The use according to claims 47 or 53, wherein the medicament is for the treatment of cancer.
- 5 55. The use according to claim 47, wherein the medicament is for the stimulation of the ability to learn and/or of the short and/or long term memory.
56. The use according to claim 47, wherein the medicament is capable of modulating proliferation and/or differentiation and/or regeneration and/or morphological
10 plasticity of cells.
57. An antibody capable of recognising and binding to an epitope comprising an amino acid sequence selected from SEQ ID NOs: 1-6.
- 15 58. A method of treating a condition or disease wherein activating FGFR is beneficial for the treatment, said method comprising administering to an individual in need a compound according to any of the claims 1-39 or a pharmaceutical composition according to claims 40-43.
- 20 59. The method according to claims 58, wherein the condition or disease is as defined in any of the claims 47-56.

1 / 11

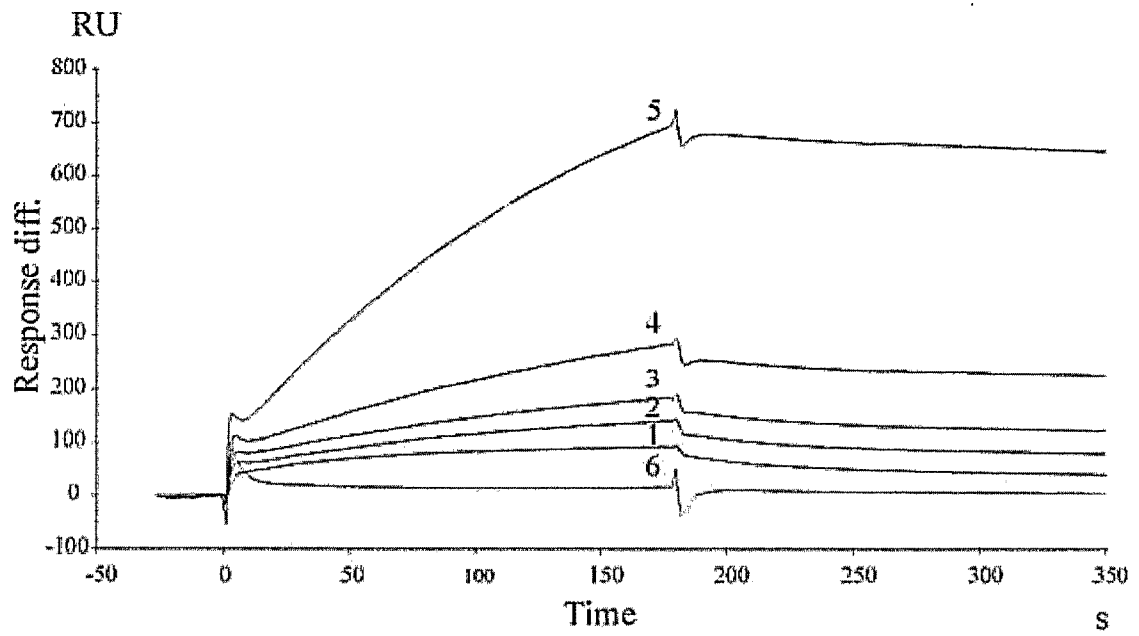


Figure 1

2 / 11

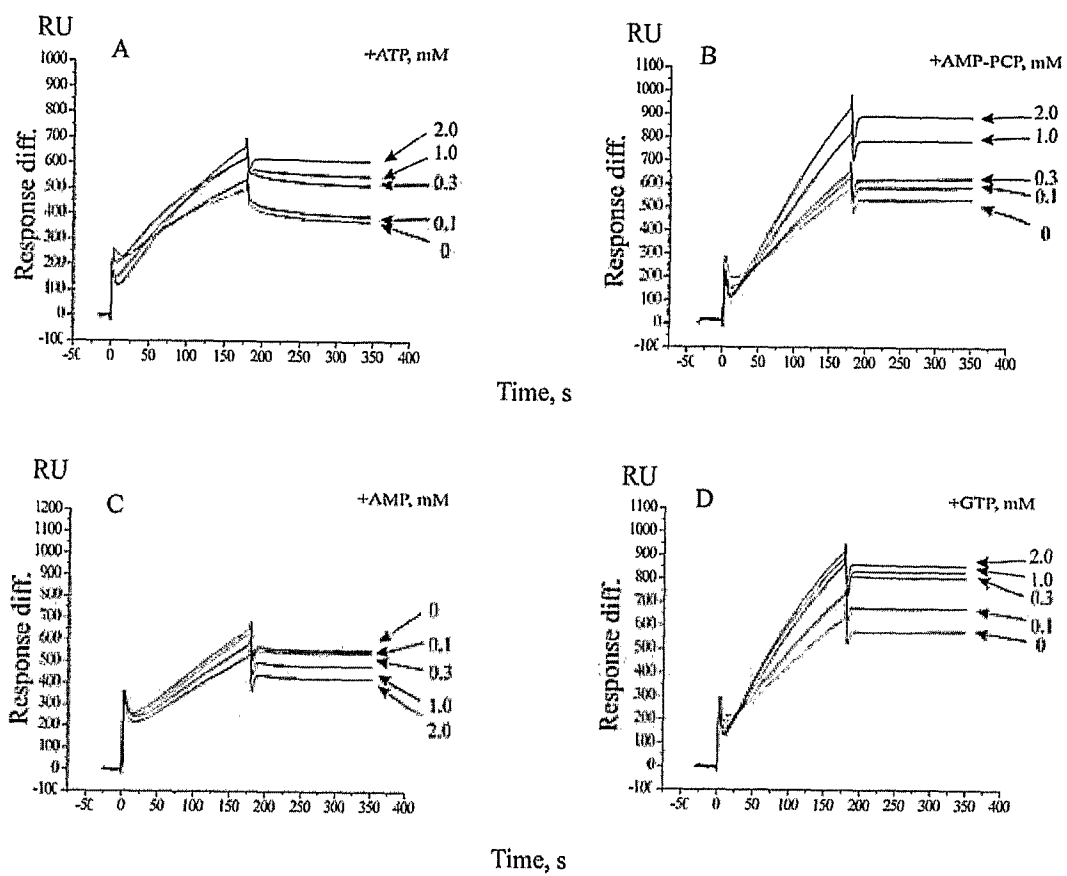
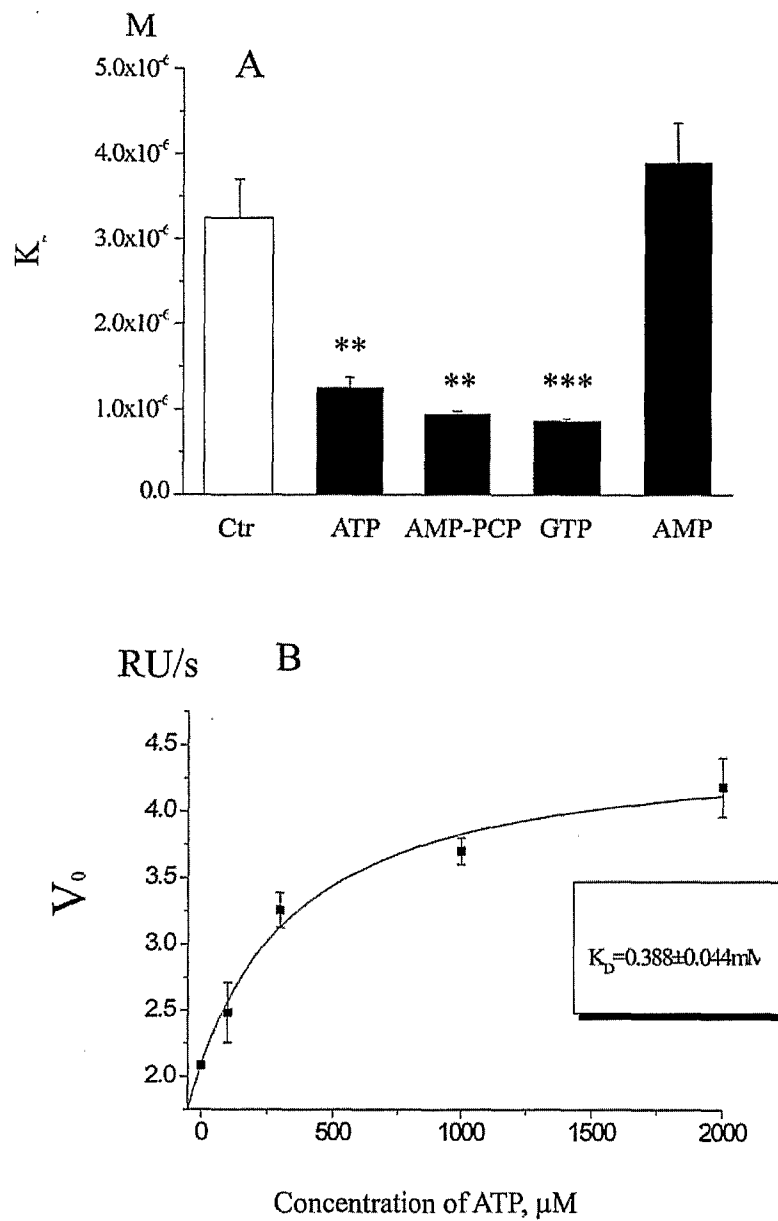


Figure 2

3 / 11

**Figure 3**

4 / 11

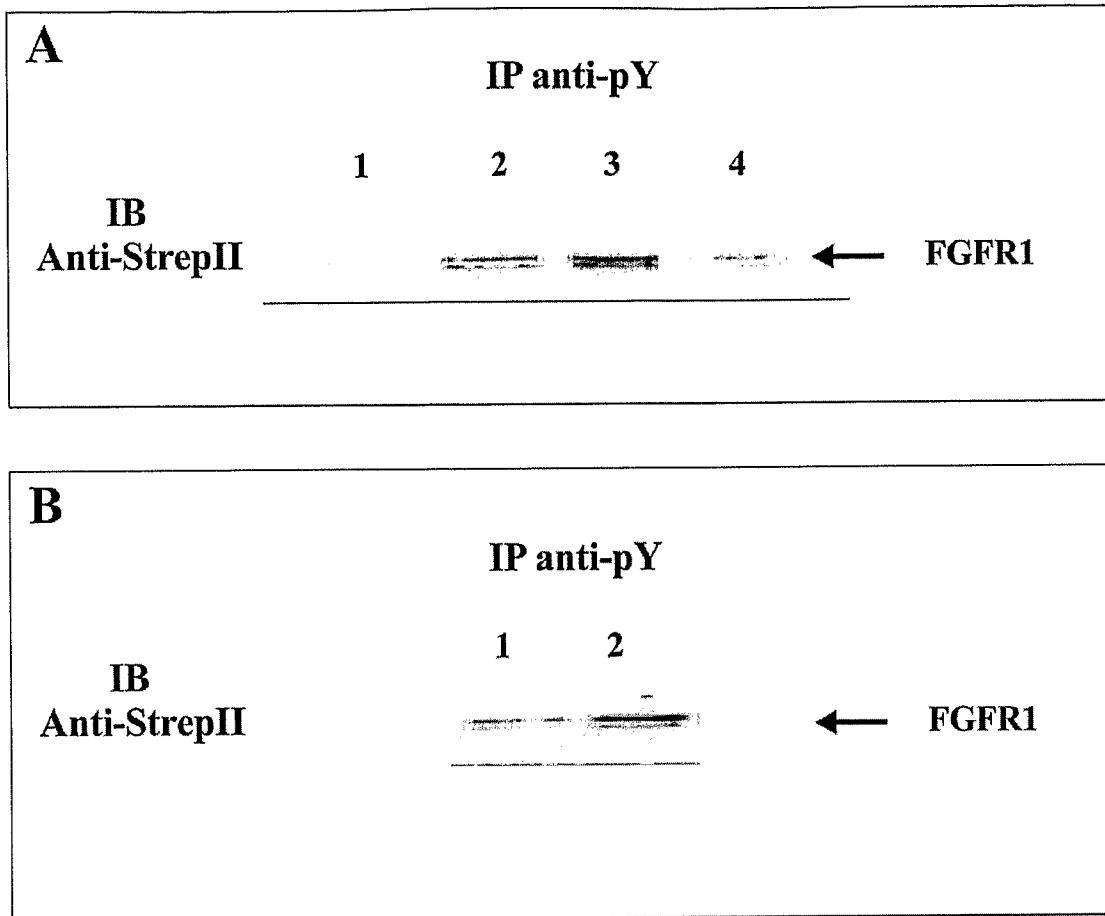


Figure 4

5 / 11

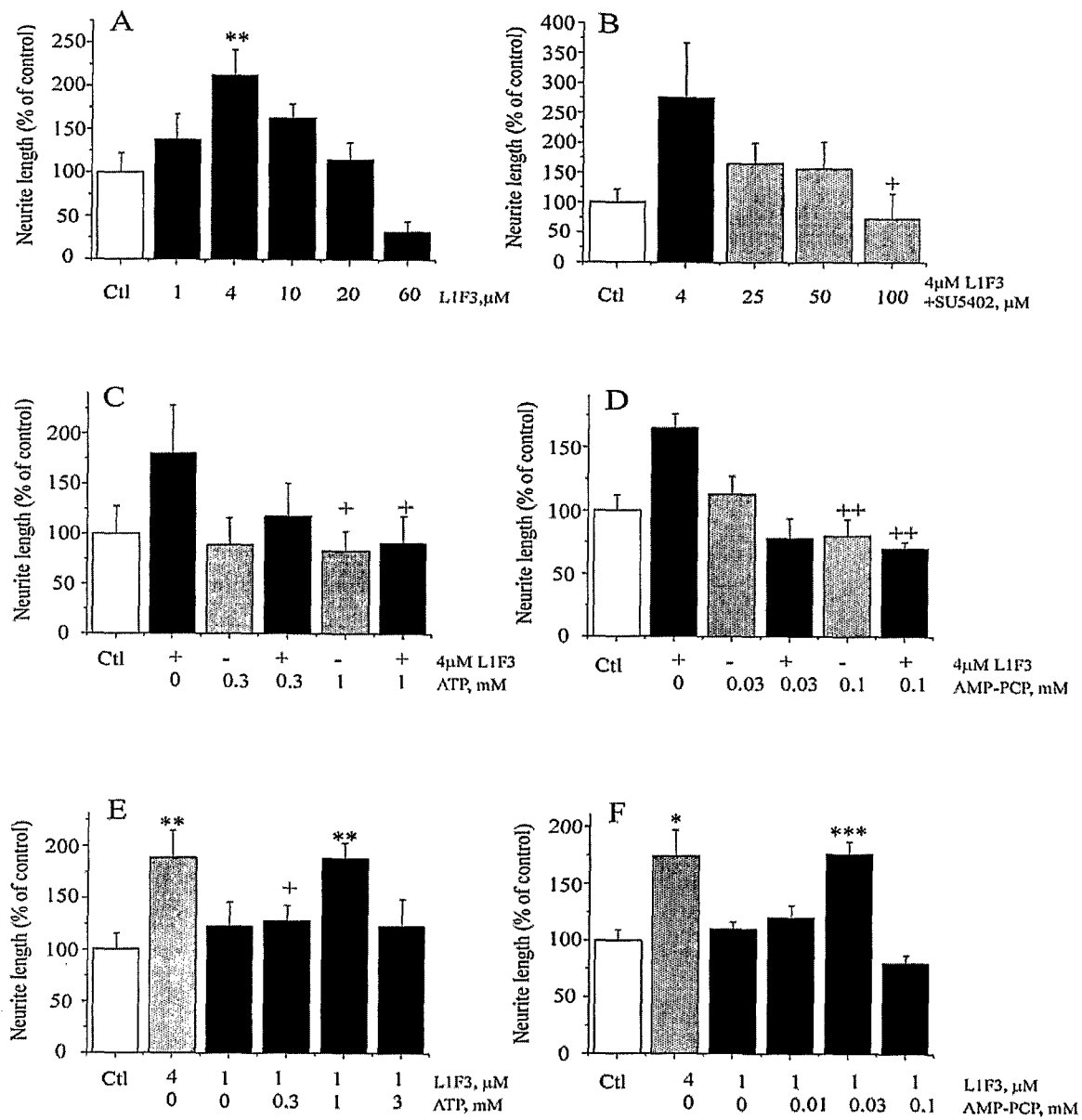


Figure 5

6 / 11

	F1ABL	F1BCL	F1CDL	F1DEL	
<u>GSPGPVPHLELSDRHLLKQSQVHLSWSPAEDHNSPIEKYDIEFEDKEMAPEKWFSLGKVP</u> <u>PGMOTSTTLKLS</u>					
F1EFL	F1FGL		F2ABL	F2BCL	
<u>PYVHYTFRVTAINKYCPGEPSPVSETVVTPEAAPEKNPVDVRGEGNETNNMVITWKPLRWMD</u> <u>WNAFQIQIR</u>					
F2CDL	F2DEL	F2EFL	F2FGL		
<u>VQMRPLGKQETWKEQTVSDPFLVVSNTSTFVPYEIKVOAVNNQ</u> <u>GKGP</u> <u>EPQVTICYS</u> <u>CEDYPQVS</u> <u>PELEDIT</u>					
	F3ABL	F3BCL	F3CDL	F3DEL	
<u>IFNSSTVLVRWRPVDLAQVKGHLRGYNVTYWNKGSQRKHSKRHVHKSHMVPAN</u> <u>TTSAILSGLE</u> <u>RPYSSYHV</u>					
F3EFL	F3FGL		F4ABL	F4BCL	
<u>EVQAFNGRGLGPASEWTFSTFEGVPGHPEALHLECQSD</u> <u>TSLLLHWQPPLSH</u> <u>NGVLTGYLLSYHPLD</u> <u>GESKE</u>					
F4CDL	F4DEL	F4EFL	F4FGL		
<u>QLFFNLSD</u> <u>FELRTENLTNLNPD</u> <u>LQYRFQLOAT</u> <u>THQCPGEAIVREGG</u> <u>IMALFGKPDFGNI</u> <u>SVTAGENYSVVS</u>					
F5ABL	F5BCL	F5CDL	F5DEL	F5EFL	F5FGL
<u>WVREGQC</u> <u>NFRFHILFKAL</u> <u>PEGKVSPDHQ</u> <u>POPOQYVS</u> <u>YNQSSYTQNDLQ</u> <u>PD</u> <u>TKYEI</u> <u>HLMREKVL</u> <u>LHHLAVK</u> <u>TNGTC</u>					

Figure 6

7 / 11

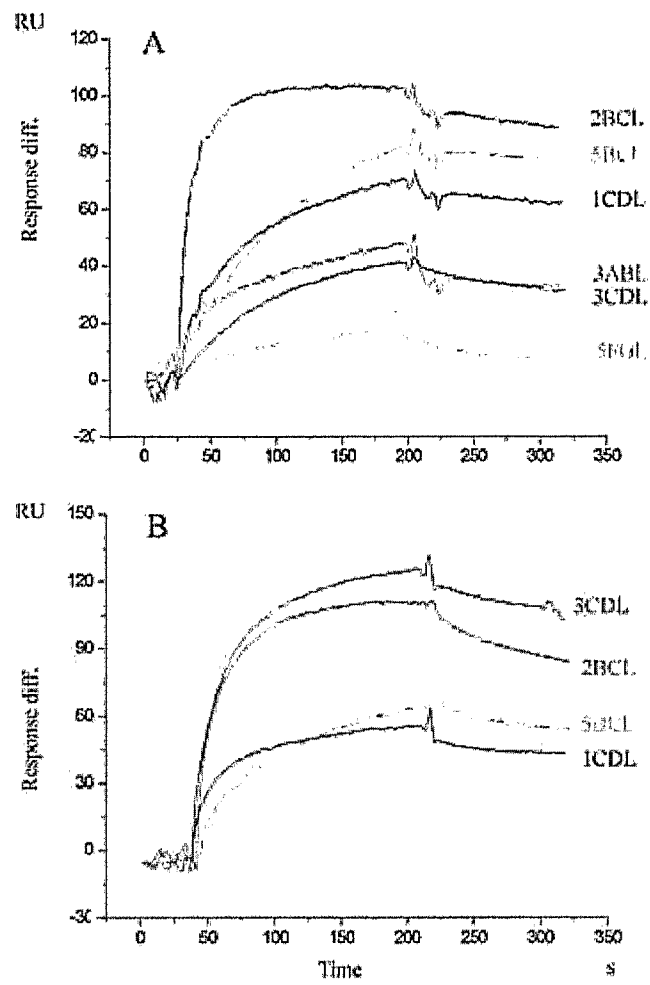


Figure 7

8 / 11

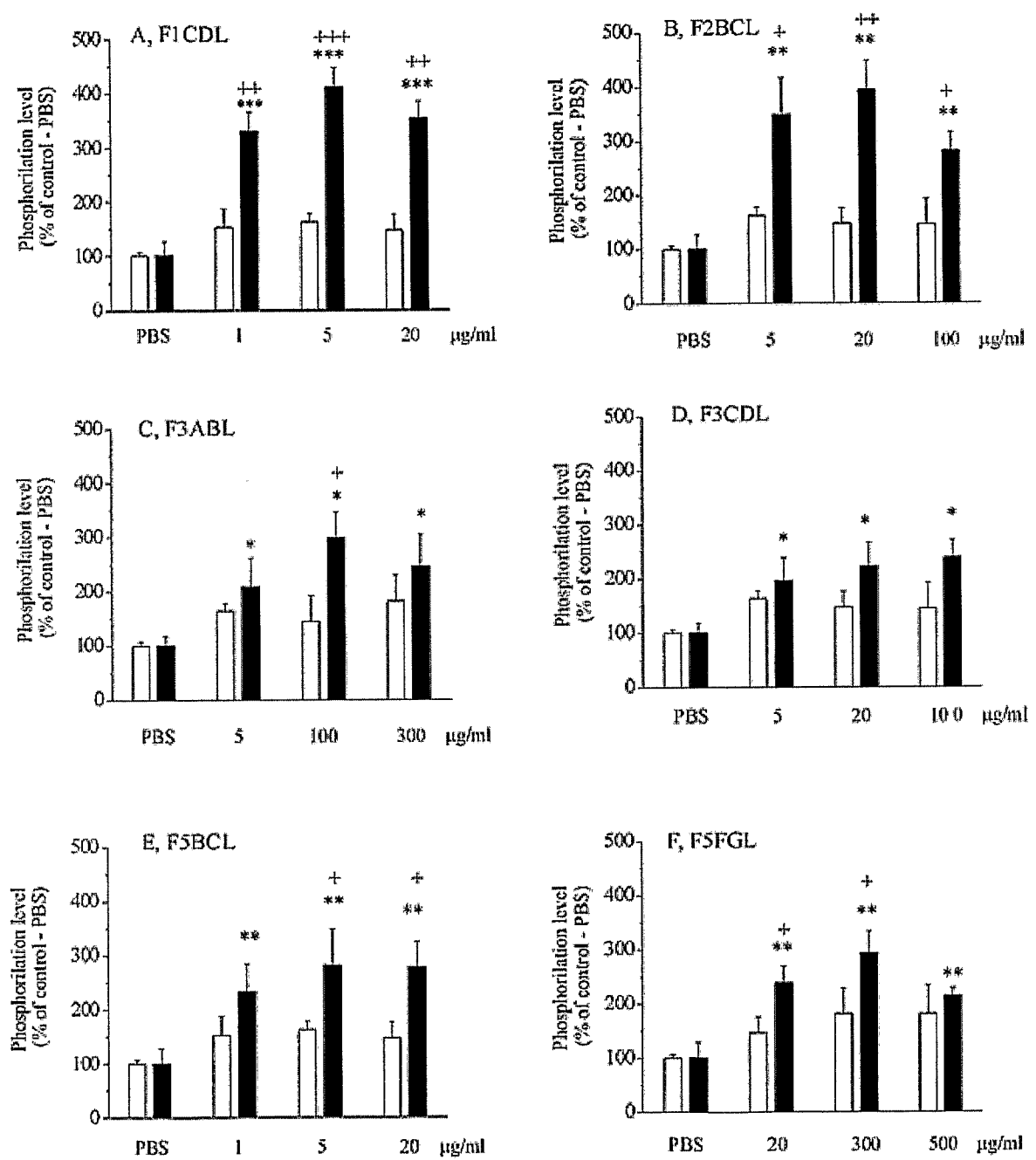


Figure 8

9 / 11

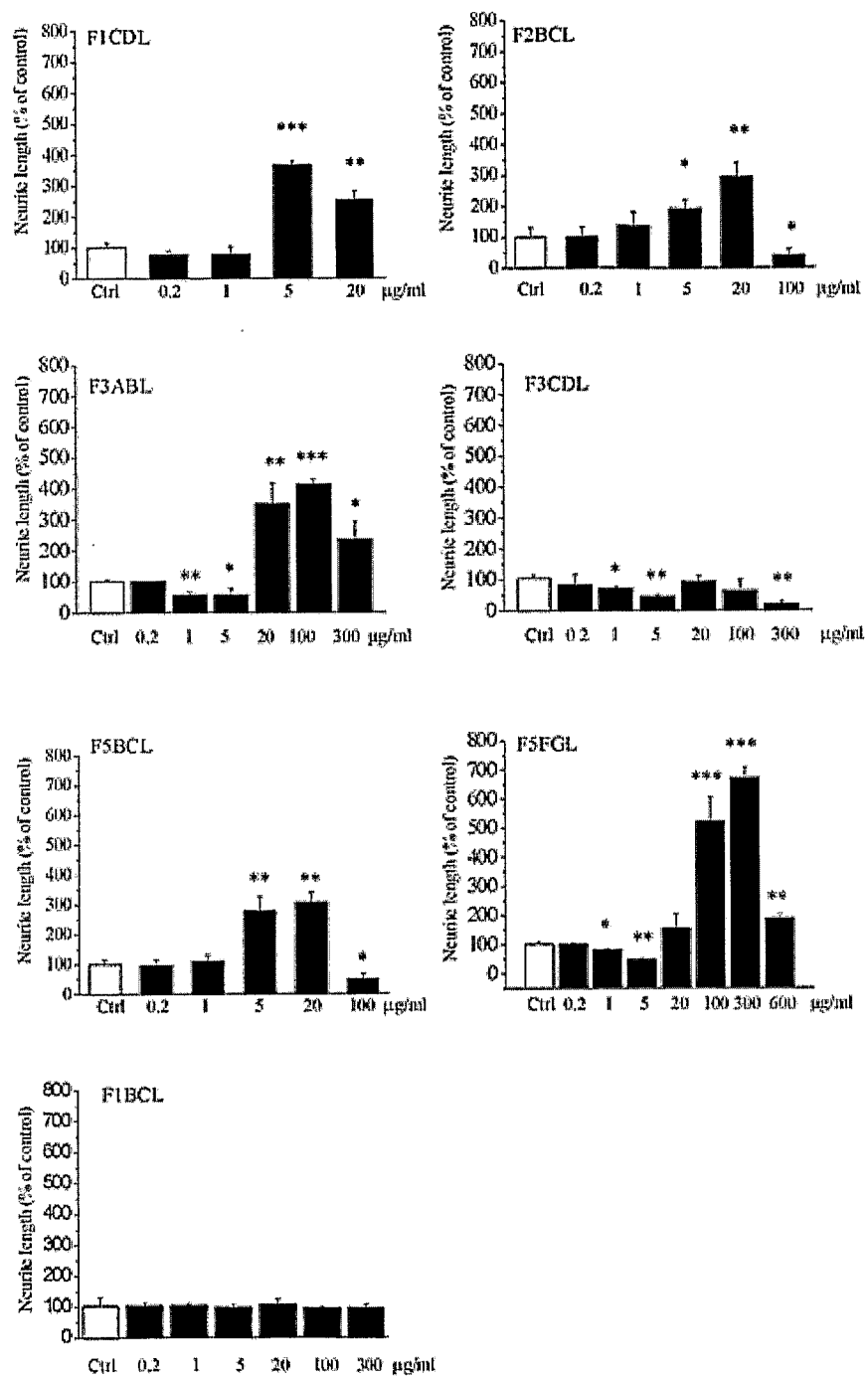


Figure 9

10 / 11

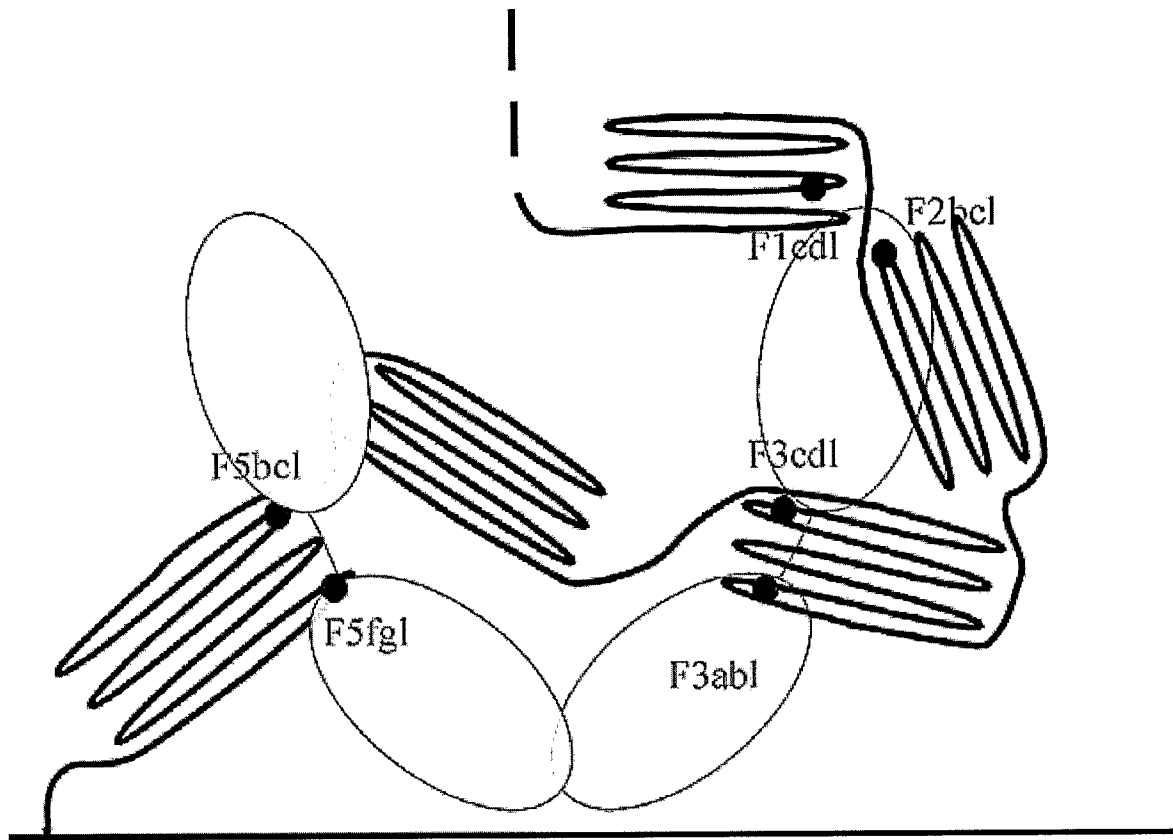


Figure 10

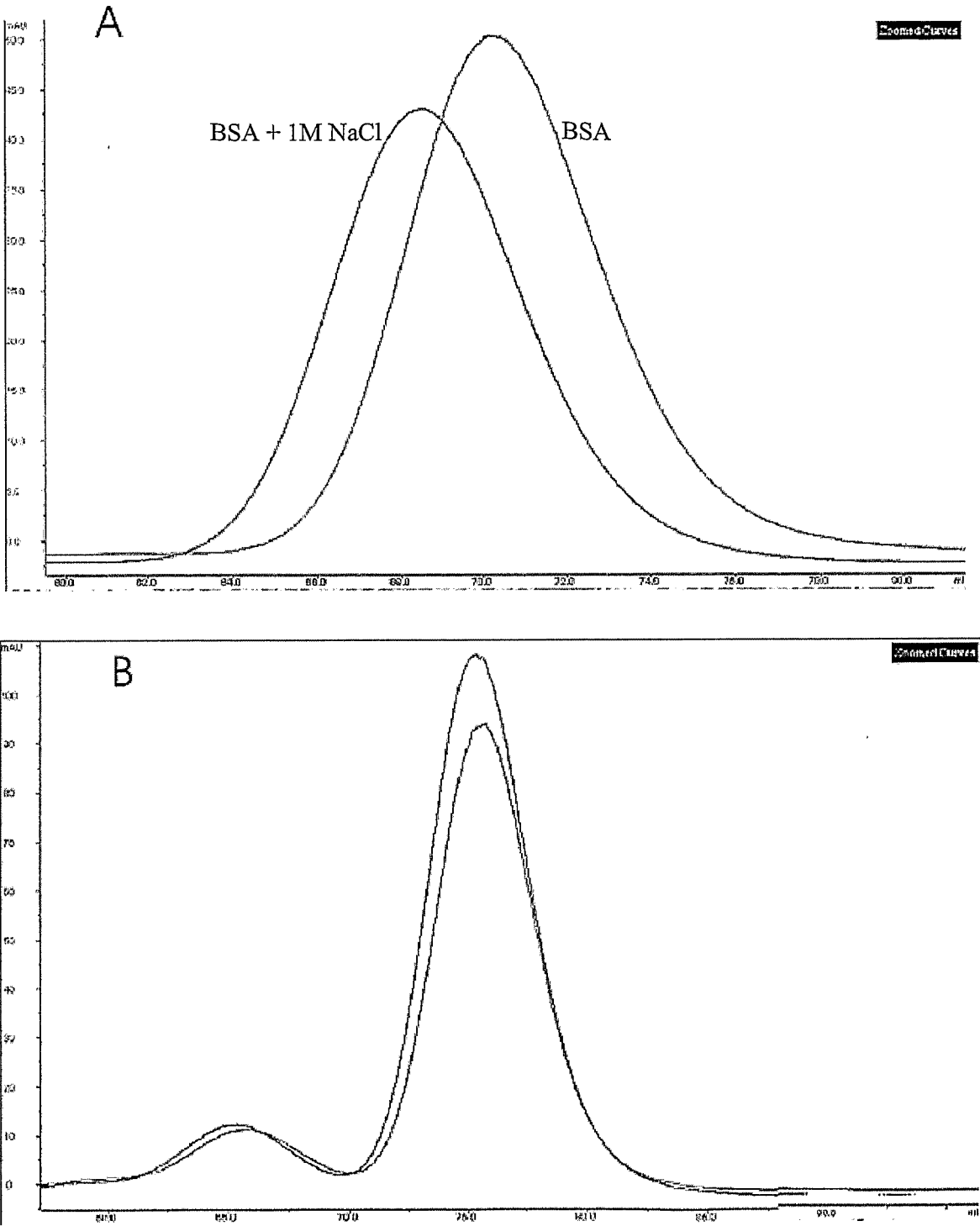


Figure 11