Attached documents

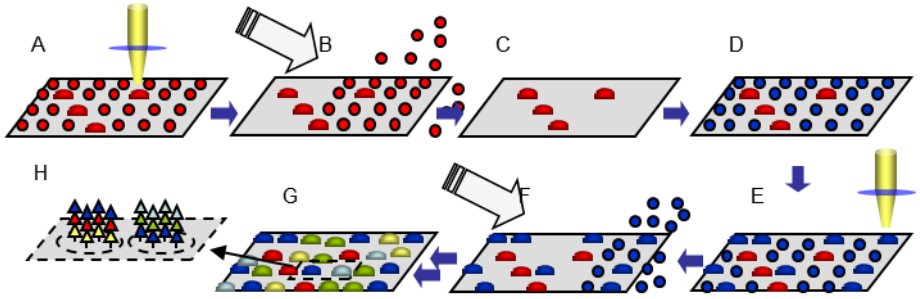

Fig. 1; *Laser-induced-melting-method.* *The array support is uniformly covered with amino acid particles (A, D). These are melted at selected areas and thereby glued to the support (A, E). Non-melted particles are blown away (B, F). Repeating this patterning with all 20 different amino acid particles (G) and with several coupling layers results into the combinatorial synthesis of a peptide array (H).*

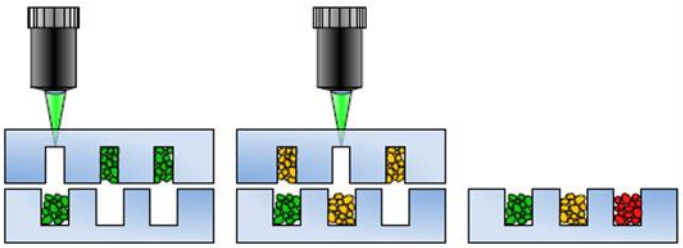

Fig. 2; *Micro structured donor glass slides are filled with particles of a first kind. A laser pulse transfers particles of a first kind from selected cavities to a synthesis slide (left), and particles of a second kind from another donor slide (middle), which results into a synthesis slide that is patterned with 20 different amino acid particles (right).*

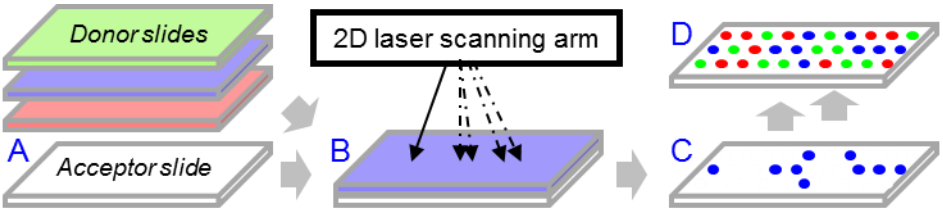

Fig. 3; *Combinatorial synthesis of a peptide array by laser-induced-forward-transfer of materials (cLIFT).* *(A) Donor slides of different amino acid building blocks (different colours) are placed on top of an acceptor slide. (B) A laser scanning system transfers tiny amounts of material to the acceptor slide. (C) Afterwards, the donor slide is removed. (D) Repeating these steps with different donor slides, results in a combinatorial pattern.*

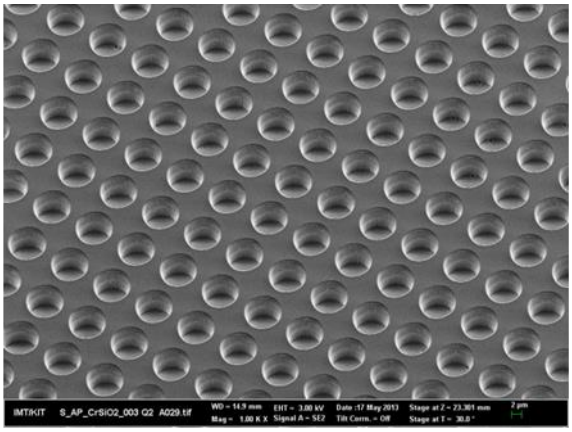

Fig. 4; *Micro structured glass slide (done by dry etching). Diameter of cavities is 7 µm, depth of cavities is 6.8 µm, and pitch of cavities is 10 µm.*
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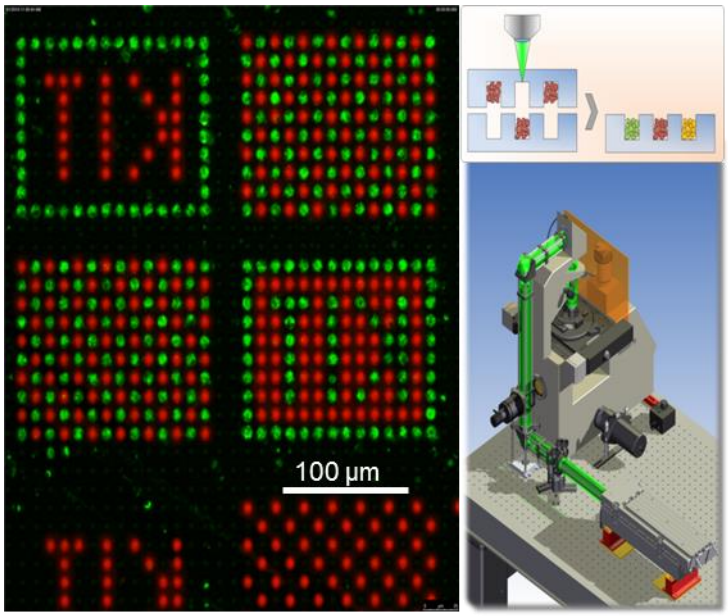

Fig. 5; ***Using microstructured glass slides to transfer amino acid particles.*** *(upper right) Microstructured glass slides are each filled with one kind of amino acid particles that are transferred with a short laser pulse to an adjacent synthesis slide. (lower right) A laser scanning system transfers amino acid particles at selected cavities to the acceptor slide. (left) Array of 1 Mio Flag- and HA-peptides.*

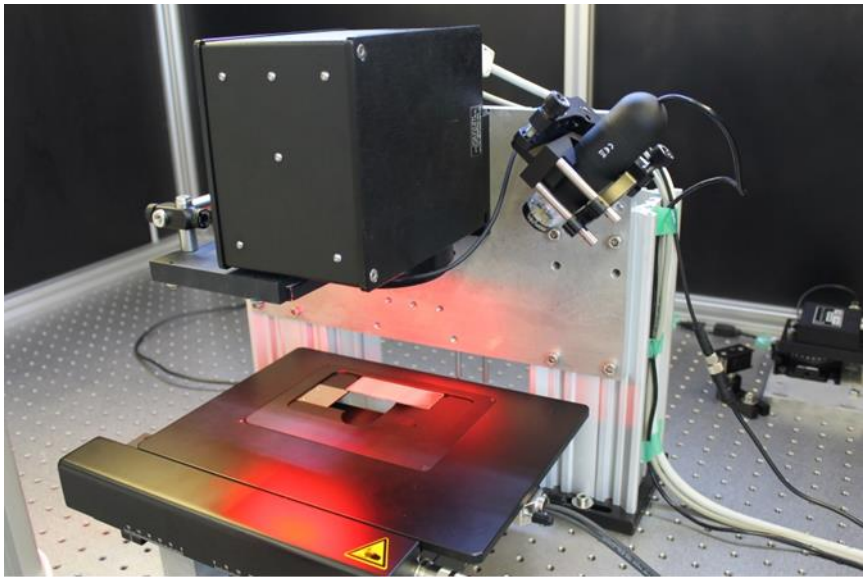

Fig. 6; *Laser system with scan head and xy-stage with substrate.*

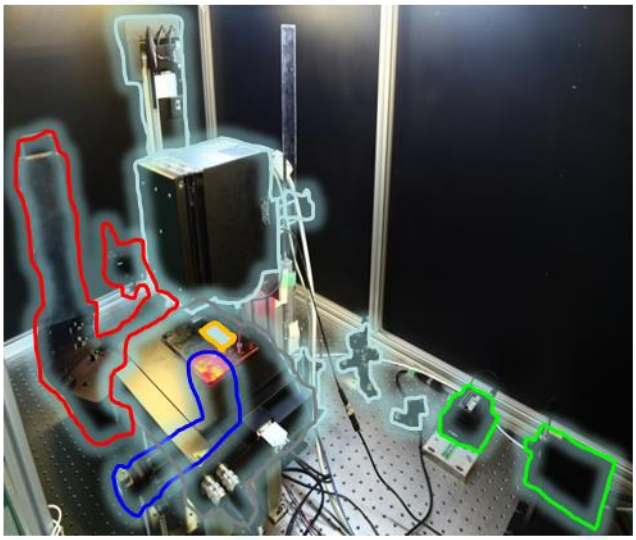

Fig. 7; *Assembled cLIFT-array-synthesizer*. *A laser (1 W, 532 nm, continuous wave) and an acoustic optic modulator generate short flashes of light that is relayed by a 2D laser scanning arm with a moving mirror inside to a glass slide covered with a foil of donor material. A slide loader is used for the automated replacement of donor foils. A moving desk and a microscope help to monitor correct functioning of the different parts.*

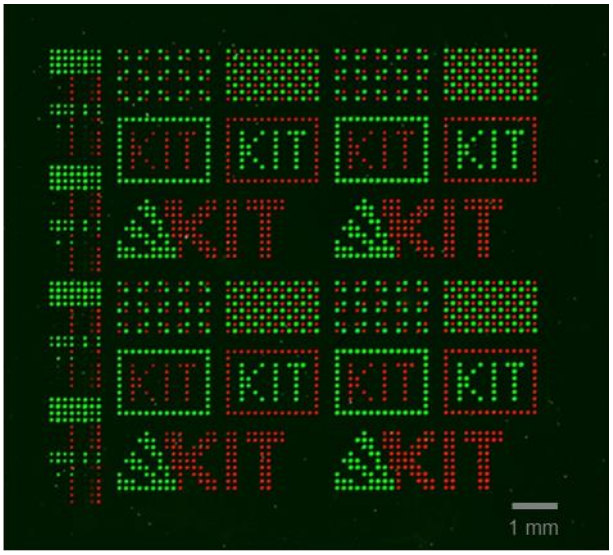

Fig. 8 ; *Combinatorial synthesis of a peptide array with 5,000 peptide spots per cm² using laser induced forward transfer (cLIFT) of different materials that comprise the different amino acid building blocks. Flag- and HA-peptides were stained with specifically binding anti-Flag- and anti-HA-antibodies.*

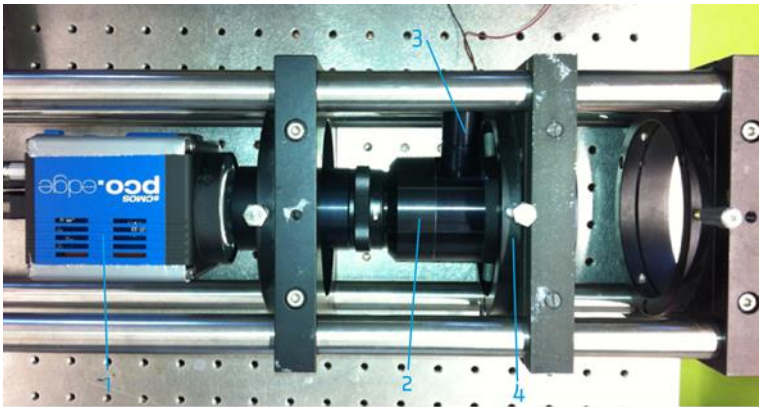

Fig. 9; *iRIfS device for massively parallel labelling-free read out of kinetic data.* The iRIfS setup consisting of (from left to right) an sCMOS camera (1), a telecentric objective (2) with the Atlas Light Engine mounted (3), and a λ/2 filter (4, hidden beneath the mounting).

	Biametrics Technology	Reference SPR (Biacore)
on-rate	(4.03 ± 0.32) x 10^4 M^{-1} s^{-1}	(4.1 ± 0.6) x 10^4 M^{-1} s^{-1}
off-rate	(3.0 ± 0.02) x 10^{-4} s^{-1}	(4.5 ± 0.6) x 10^{-5} s^{-1}
affinity	(7.14 ± 0.59) nM	(1.1 ± 0.2) nM

Fig. 10; *Kinetic data measured with Biametrics' iRIfS device vs. Biacore.* BIA's iRIfS setup measures similar affinity constants when compared to measurements theat were done with the Biacore.

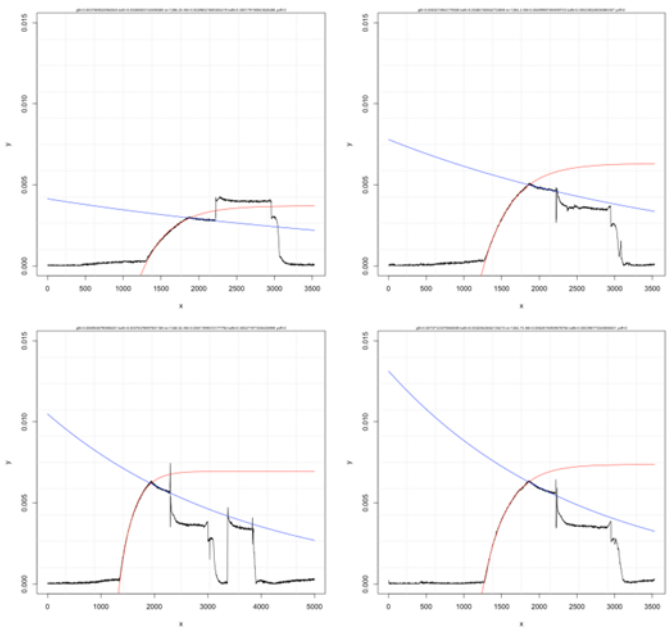

Fig. 11; ***Kinetic data from automated fitting signals measured with Biametrics' iRIfS device***. R performs the automatic fitting of association (red line) and dissociation (blue line) of the 4 binding curves of spot 9 of the PEPperPRINT array obtained by the 4 different concentrations.

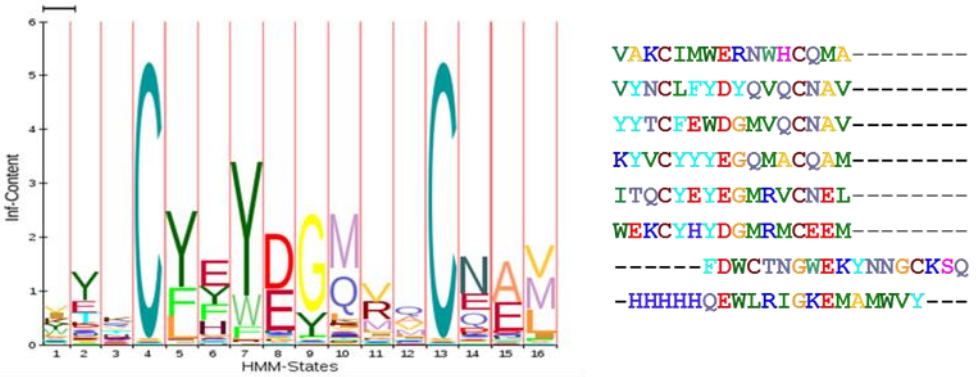

Fig. 12; ***Peptide binders to TNF superfamily targets***. Sequence alignment and logo representation of positive ELISA clones specific for the members of the TNF receptors family.

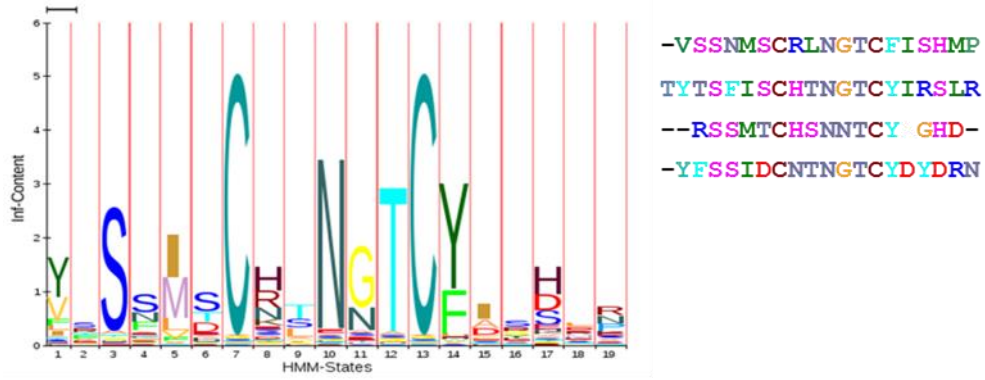

Fig. 13; ***Peptide binders to the human Fc fragment***. Sequence alignment and logo representation of positive ELISA clones specific for the for the human Fc fragment.

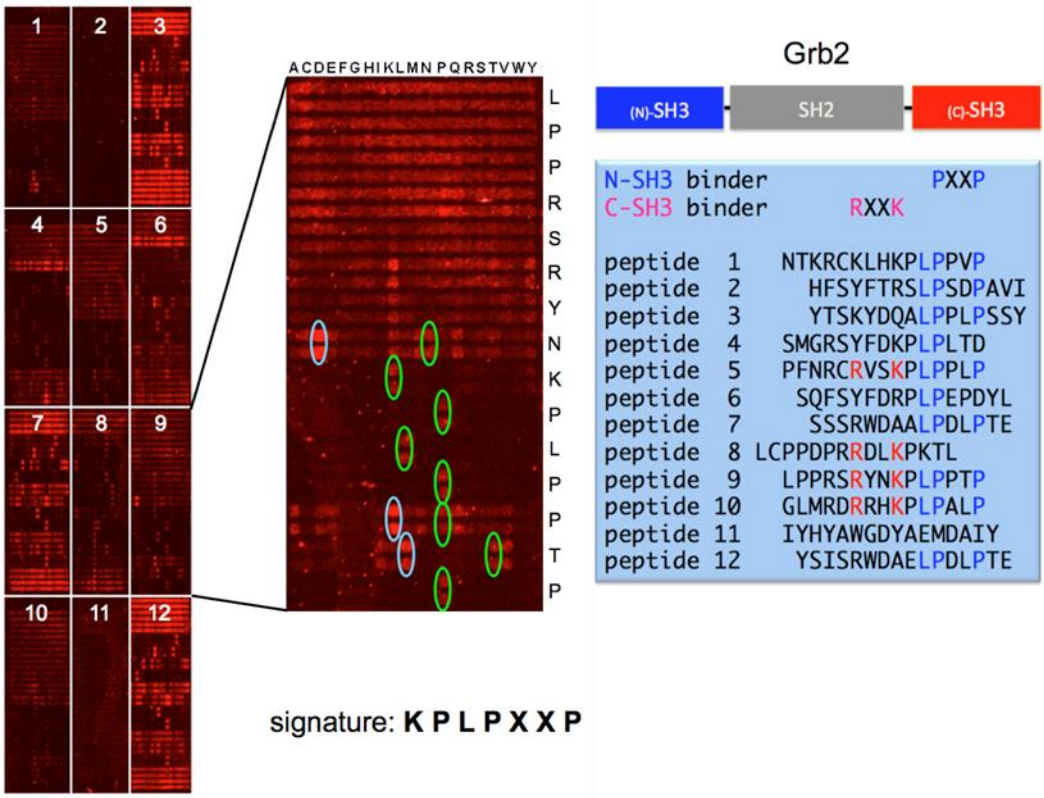

Fig. 14; ***Grb2 SH2 domains bind to clearly defined "signatures"***. Substitution analysis of Grb2 binding peptides: 12 out of 100 top binding peptides (left) were subjected to substitution analysis by exchanging every amino acid position for the remaining 19 proteinogenic amino acids. Array was stained with GST-Grb2-biotin/streptavidin-DyLight680. Binding signature of peptide 9 is indicated with green circles (KPLPXXP). Positions leading to increased signal intensity are labelled in blue. List of peptides that either bind to the C-terminal (signature RXXK) or the N-terminal (signature PXXP) SH3 domain from Grb2 (right).

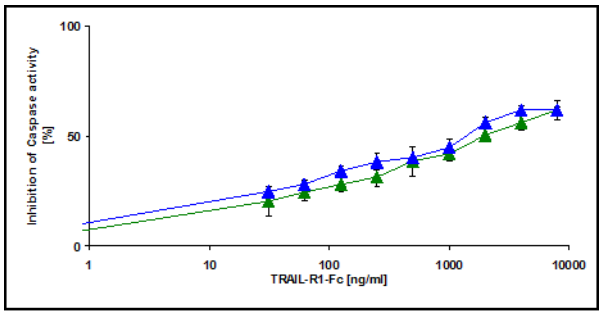

Fig. 15; *The fusion protein TRAIL-R1-Fc is biologically active. Both TRAIL-R1-Fc with and without biotin label (green and blue data points) inhibit caspase activity within Colo205 tumour cells that were treated with caspase-activating ligand TRAIL at a lethal dose.*

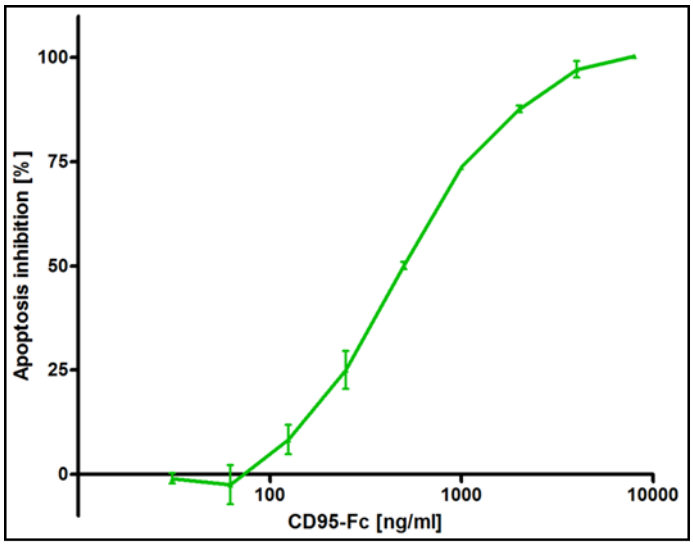

Fig. 16; *The fusion protein CD95-Fc is biologically active.* Colo205 tumour cells were treated with the caspase-activating and therefore pro-apoptotic ligand CD95L (Fas) at a lethal dose. When being co-incubated with varying amounts of CD95-Fc, caspase activity was decreased. This indicated that the fusion protein bound its soluble ligand CD95L, thus preventing it from triggering CD95 receptors on the surface of Colo205 cells in turn leading to caspase inhibition. These results establish the biological activity of the CD95-Fc fusion protein produced and deem it fit-for-purpose in a peptide array screen.

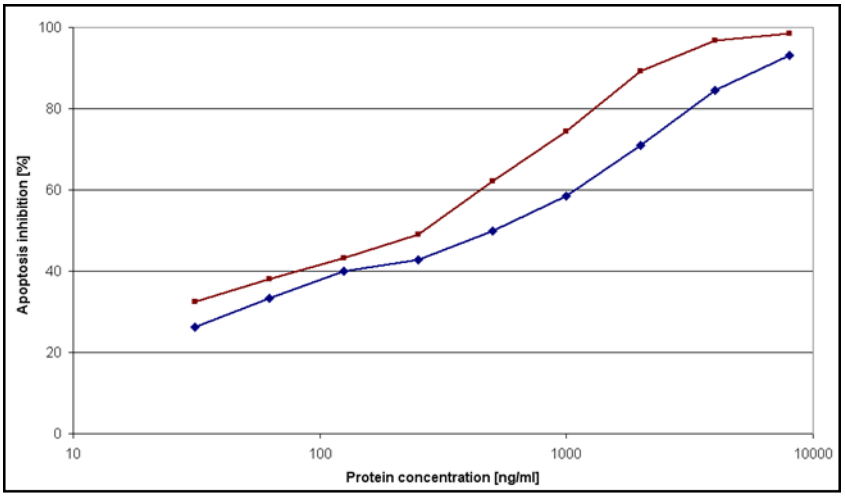

Fig. 17; *The fusion protein TRAIL-Receptor 2-Fc is biologically active and biotinylation does not interfere with biological activity.* Shown here are potency assay results for TRAIL-Receptor 2-Fc (blue curve) and TRAIL-Receptor 2-Fc-Biotin (red curve). The assay was performed on TRAIL-sensitive Colo205 cells using a TRAIL mimic as pro-apoptotic stimulus. Dose-dependent inhibition of apoptosis was visualized using a fluorescent substrate for caspase 3, which is a critical factor in the extrinsic apoptosis pathway.

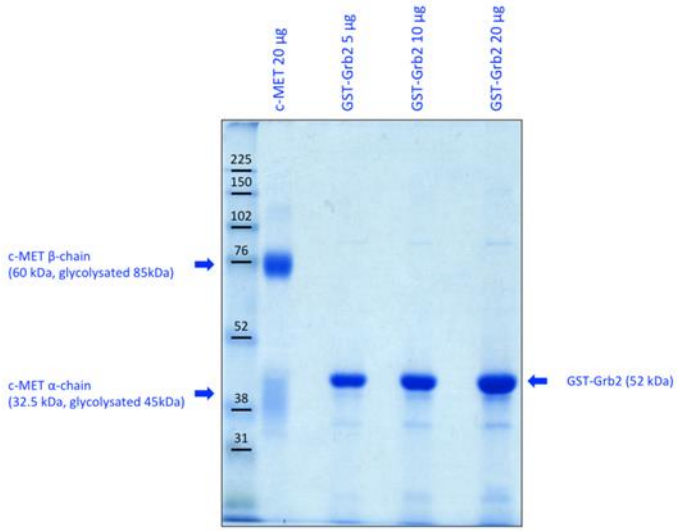

Fig. 18; ***Purity of the extracellular c-MET domain.*** The recombinantly expressed extracellular domain of c-MET (Glu 25 – Thr 932) from HEK293 cells was separated by SDS-PAGE and stained with Instant Blue dye. For comparison increasing amounts of recombinant GST-Grb2 protein were loaded. The gel shows two bands at 45 kDa and 85 kDa corresponding to two glycosylated chains of the purified protein. The purity of the protein is around 92%.