Supplementary Information

to

Rational design of a peptide capture agent for CXCL8 based on a model of the CXCL8:CXCR1 complex

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Keywords: Rational design, peptide, CXCL8, capture-agent, chemokine, GPCR, CXCR1, docking, receptor-ligand interaction, neutrophil migration

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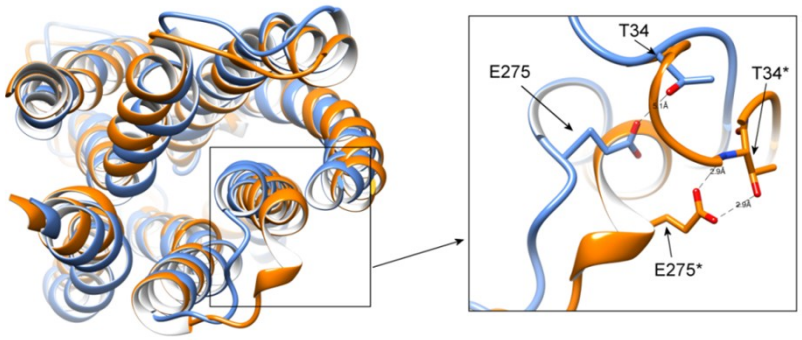

Figure S1 Conformational change in CXCR1[1] upon binding to CXCL8 in a flexible protein-protein docking from inactive (blue) to 'active-like' state (orange). The binding of CXCL8 induced a tilting of transmembrane helix TMH 7, a movement consistent with the activation of other GPCRs.[2] Only in the 'active-like' state (orange) E275 forms two stable hydrogen bonds with T34 at the N-terminus (see zoom).Hébert *et al*. found that E275 is an important residue for CXCL8:CXCR1 interaction.[3] When E275 was replaced by alanine, binding of radiolabelled CXCL8 to CXCR1 on HEK 293 cells was no longer observed. Since a two-step mechanism is proposed for the binding and activation of CXCR1 which involves interactions of several amino acids at two different sites of the receptor, this drastic influence of one residue change seems surprising. This could indicate that without E275 the receptor can no longer form a stabilized active state and thus can no longer bind CXCL8.

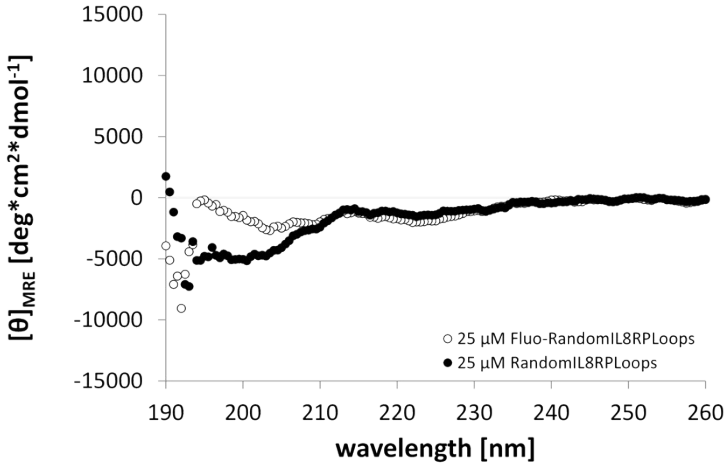

Figure S2 Circular dichroism spectra of 25 µM unlabelled randomIL8RPLoops (LWMIVRKAR-Ahx-RAMQLTDT) and labelled Fluo-randomIl8RPLoops peptide (Fluo-LWMIVRKAR-Ahx-RAMQLTDT) in *ls*-PBS. The randomized peptide shows no pronounced tendency to form helices with no minima at 222 nm and no maxima at 190 nm.

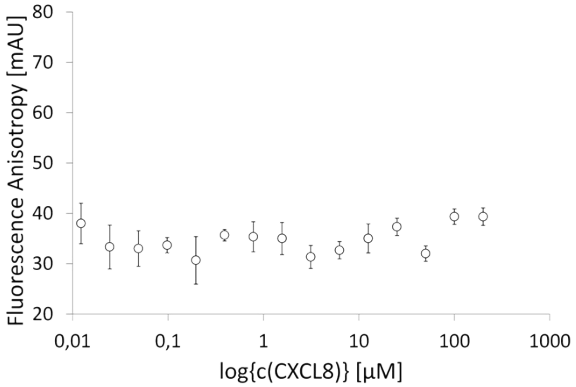

Figure S3 Fluorescence anisotropy measurement with randomized Fluo-IL8RPLoops peptide (Fluo-LWMIVRKAR-Ahx-RAMQLTDT) in different concentrations of CXCL8 in *ls*-PBS. The randomized peptide sequence shows no affinity for CXCL8.

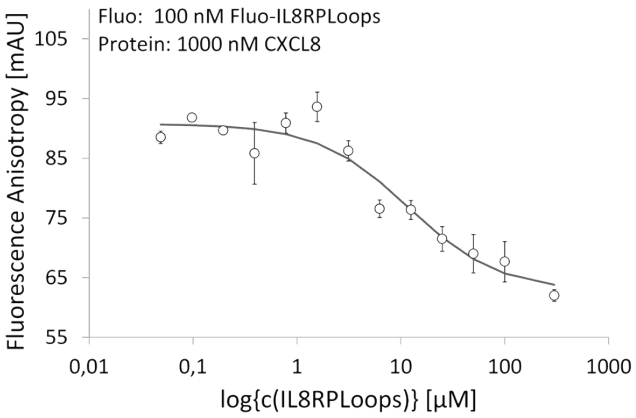

Figure S4 Fluorescence Anisotropy competition data, 100 nM Fluo-IL8RPLoops (in 1 µM CXCL8) was displaced by unlabelled IL8RPLoops. f = min+(max-min)/(1 + 10^(x-logIC50)), calculated with SigmaPlot. Four individual measurements gave an IC_{50} value of 43.9 ± 3.7 µM. The graph shows a single experiment with STD. According to Moerke[4] for a competition experiment a receptor concentration should be chosen that produces 50 % to 80 % increase in fluorescence anisotropy/polarization between the free ligand and the completely bound state. Rossi and Taylor[5] also state that a protein concentration that gives > 50 % of bound labelled ligand is a good compromise between saving material and achieving an acceptable dynamic range. Also, in order to prevent receptor depletion, no more than 10 % of the receptor molecules should be bound in the complex. The starting conditions of the experiment were chosen to meet these recommendations: According to the standard formula of receptor-ligand binding the amount of receptor-ligand complex at equilibrium conditions is calculated by RL=(R0+L0+Kd)/2-sqrt(((R0+L0+Kd)^2)/4-(R0*L0)) with RL: concentration of receptor-ligand complex, R0/L0: total concentration of receptor/ligand. At the conditions described, L0=100 nM Fluo-IL8RPLoops and R0=1000 nM CXCL8 and Kd=500 nM there are 65,15 % (RL/L0) of the ligand bound at equilibrium and 6,52 % (RL/R0) of the receptor bound at equilibrium.

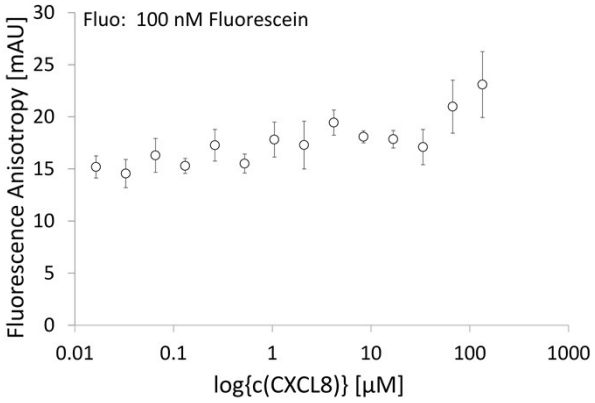

Figure S5 Fluorescence Anisotropy measurement of the interaction of fluorescein (100 nM, sodium salt) with CXCL8 wild type (wt) in *ls*-PBS. The experiment shows that fluorescein has very low affinity for CXCL8 (K_d > 100 µM).

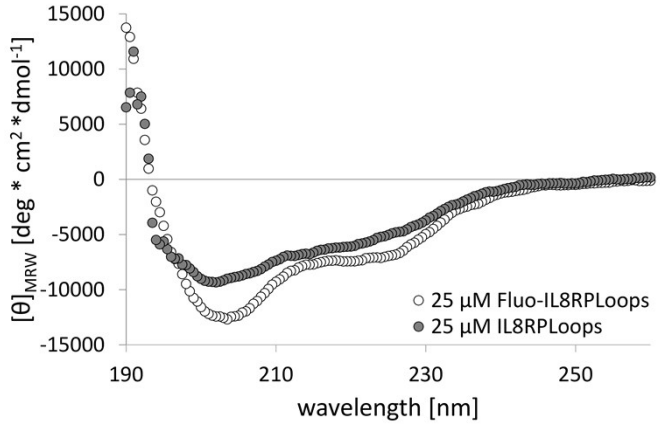

Figure S6 Circular dichroism spectra of 25 µM unlabelled IL8RPLoops and 25 µM Fluo-IL8RPLoops in *ls*-PBS. Unlabelled IL8RPLoops shows the same yet less distinct characteristic minima as labelled IL8RPLoops. The competition experiment (see Figure S2) shows that the fluorescein labelled peptide has a higher affinity for CXCL8. Taken together, these results suggest that the fluorescence label most likely stabilizes the peptide fold.

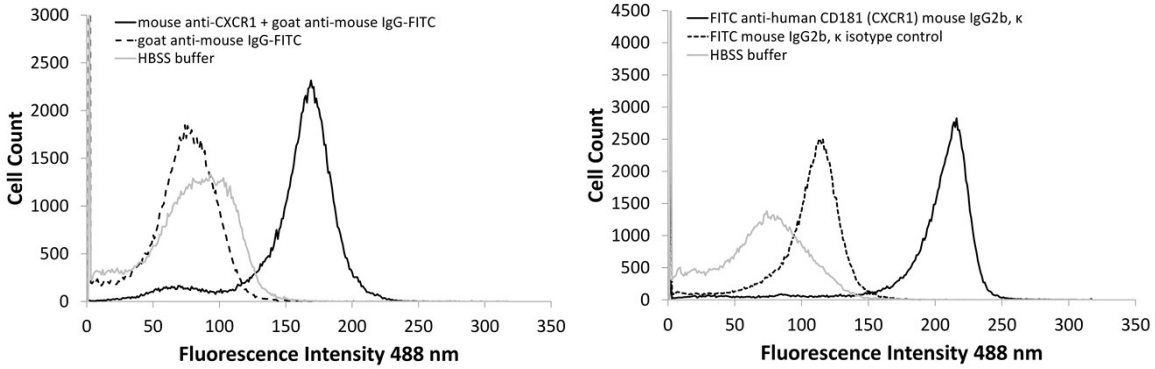

Figure S7 FACS data of HEK293 cells stably transfected with CXCR1, detected by two types of antibody. 100000 cells were counted per experiment. Left: HEK293 cells were incubated with mouse monoclonal anti-CXCR1 (Abcam, ab60254) primary antibody and goat anti-mouse IgG-FITC (Santa Cruz, sc2010) secondary antibody in HBSS buffer (Hank's Balanced Salt Solution). When incubated with secondary antibody alone, no unspecific interactions could be detected (dotted black line). When incubated with primary antibody and consecutively with secondary antibody, cells showed a significantly increased amount of fluorescence in the FITC channel (black line). Right: cells incubated with primary labelled antibody FITC-anti-human-CD181 (BioLegend #320606) showed significantly increased fluorescence (black line). The isotype control FITC mouse IgG2b, κ (BioLegend #400310) showed only minor unspecific interactions with the HEK293 cells.

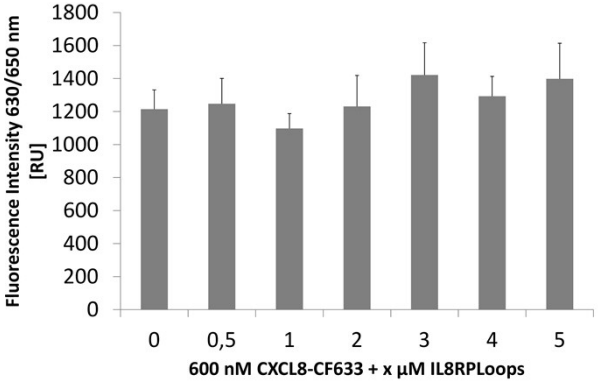

Figure S8 No quenching of CXCL8-CF633 fluorescence (ex 630 nm, em 650 nm) occurs upon mixing with IL8RPLoops peptide. CXCL8-CF633 was mixed with IL8RPLoops to give final concentrations of 600 nM CXCL8-CF633 with 0.5 µM, 1.0 µM, 2.0 µM, 3.0 µM, 4.0 µM, 5.0 µM IL8RPLoops. No significant changes in the fluorescence intensity were observed.

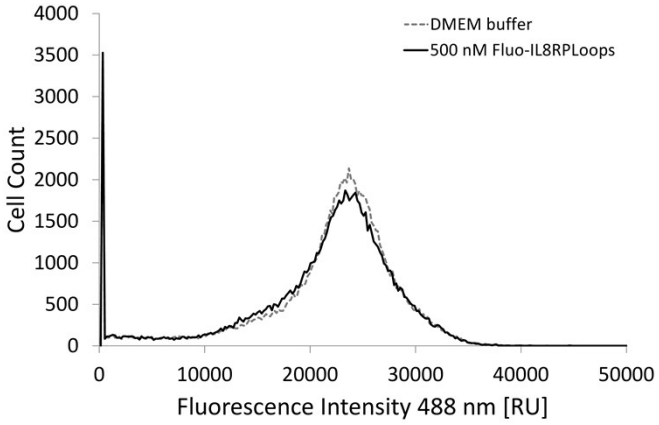

Figure S9 Fluorescence intensity at 488 nm of the FACS experiment with fluorescence labelled IL8RPLoops. Cell fluorescence is not shifted upon treatment with 500 nM Fluo-IL8RPLoops which indicates that there are no unspecific interactions of Fluo-IL8RPLoops with the HEK 293 cells.

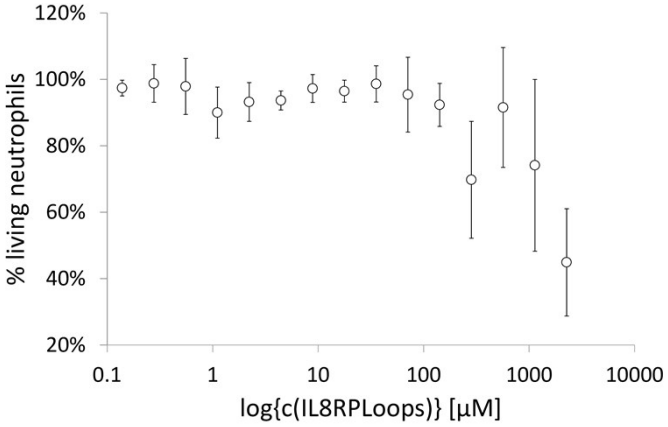

Figure S10 Cell viability assay with human neutrophil granulocytes treated with different concentrations of IL8RPLoops. The peptide shows no toxic effect in the concentration range relevant for the conducted *in vivo* assays.

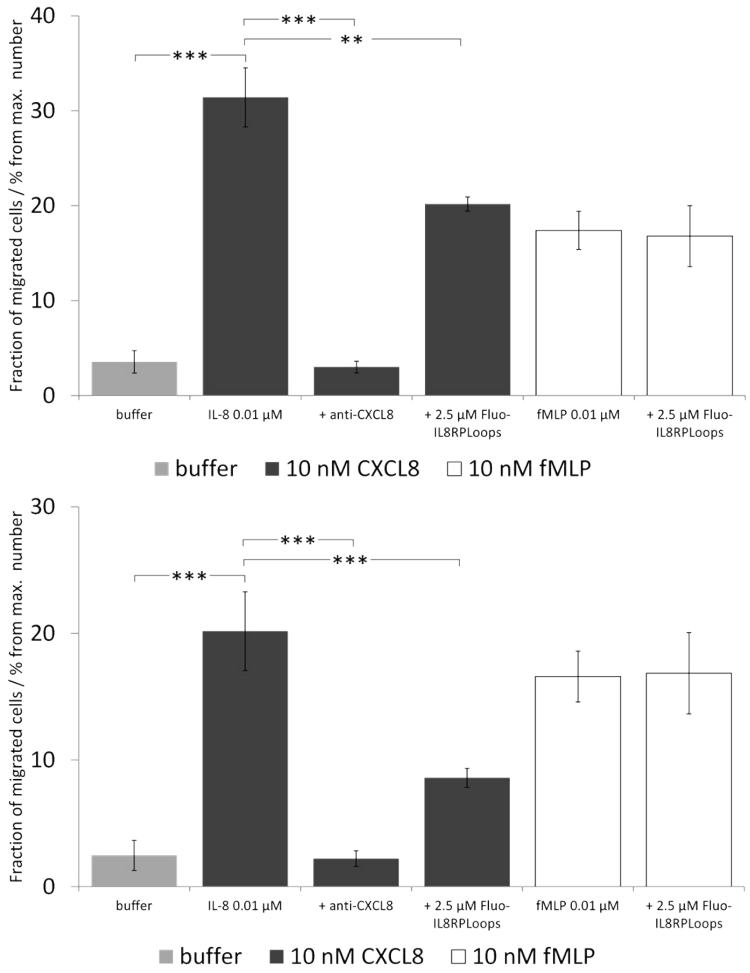

Figure S11 Neutrophil migration assay with human neutrophil granulocytes. Shown are two unrelated experiments with cells from independent donors. Error bars represent STD. Statistical differences between the CXCL8 treated cells and the cells treated with CXCL8 and inhibitors are indicated (***$P < 0.001$). Negative control (buffer, light gray): chemotaxis buffer (RPMI medium with 0.2 % bovine serum albumin BSA). Positive control: 10 nM CXCL8 in chemotaxis buffer (dark gray) and 10 nM fMLP in chemotaxis buffer (white). Inhibitor control: chemoattractant with anti-CXCL8 mouse monoclonal antibody. fMLP induced migration is not influenced.

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