Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

A fluorescence polarization assay for the experimental validation of an *in silico* model of the chemokine CXCL8 binding to receptor derived peptides

Dr. Maria Girrbach [a],[b], Dr. Irene Meliciani[c], Dipl.-Biotechnol. Björn Waterkotte[b],[d], Dipl.-Ing. (FH) Susann Berthold[e], Alisha Oster[b], Fiona Brurein[b],[d], Dipl. phys. Timo Strunk[c], Dr. Parvesh Wadhwani[f], Prof. Dr. Sonja Berensmeier[e], Priv.-Doz. Dr. Wolfgang Wenzel[c], Prof. Dr. Katja Schmitz[b],[d],[g],*

*Karlsruhe Institute of Technology
Centre for Functional Nanostructures
Haid-und-Neu-Straße 6
76131 Karlsruhe, Germany

[b]Karlsruhe Institute of Technology
Institute of Functional Interfaces
Hermann-von-Helmholtz-Platz 1
76344 Eggenstein-Leopoldshafen, Germany

[c]Karlsruhe Institute of Technology
Institute of Nanotechnology
Hermann-von-Helmholtz-Platz 1
76344 Eggenstein-Leopoldshafen, Germany

[d]Karlsruhe Institute of Technology
Institute of Organic Chemistry
Fritz-Haber-Weg 6
76131 Karlsruhe, Germany

[e]Technische Universität München
Bioseparation Engineering Group
Boltzmannstr. 15
85748 Garching, Germany

[f]Karlsruhe Institute of Technology
Institute of Biological Interfaces (IBG-2)
Hermann-von-Helmholtz-Platz 1
76344 Eggenstein-Leopoldshafen, Germany

[g]Technical University of Darmstadt
Clemens-Schöpf-Institut für Organische Chemie und Biochemie
Alarich-Weiss-Str. 4
64287 Darmstadt, Germany

*To whom correspondence should be addressed

Tel.: +49-6151-16-6964
Fax: +49 6151 16-72058
E-mail: schmitz@biochemie.tu-darmstadt.de

Supporting Information

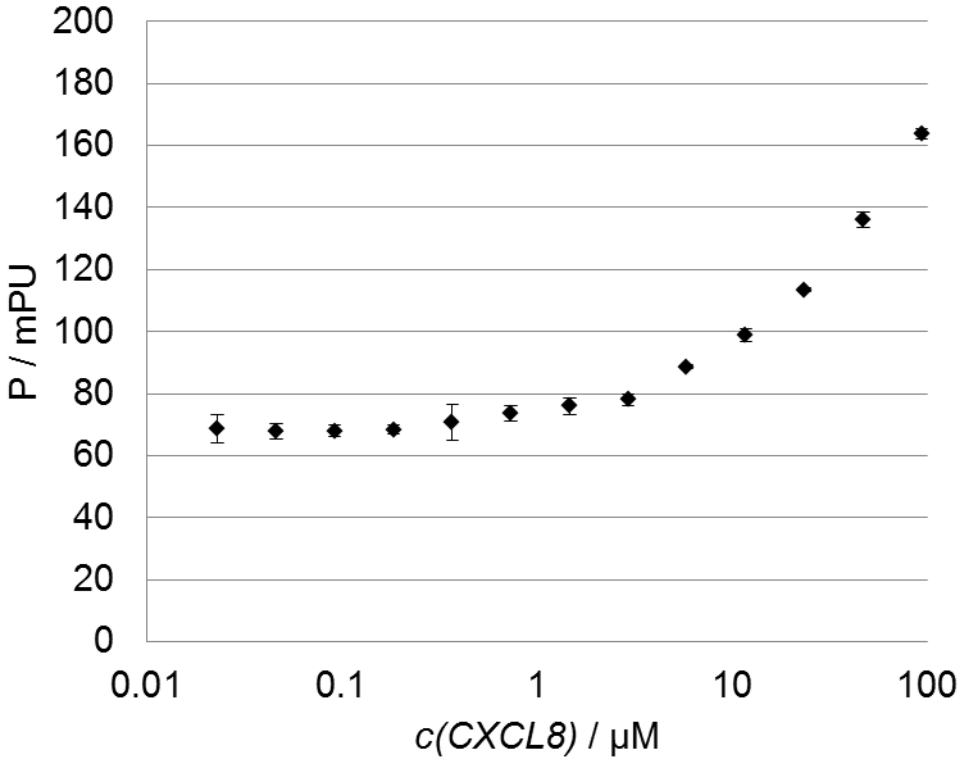

Figure S1: Initial experiments with IL-8 and fluorescein-labeled receptor peptide with aminohexanoic acid (Ahx) instead of glycine 7 in PBS with 0.1% Tween, pH 7.4. Error bars indicate standard deviation of 4 replicates.

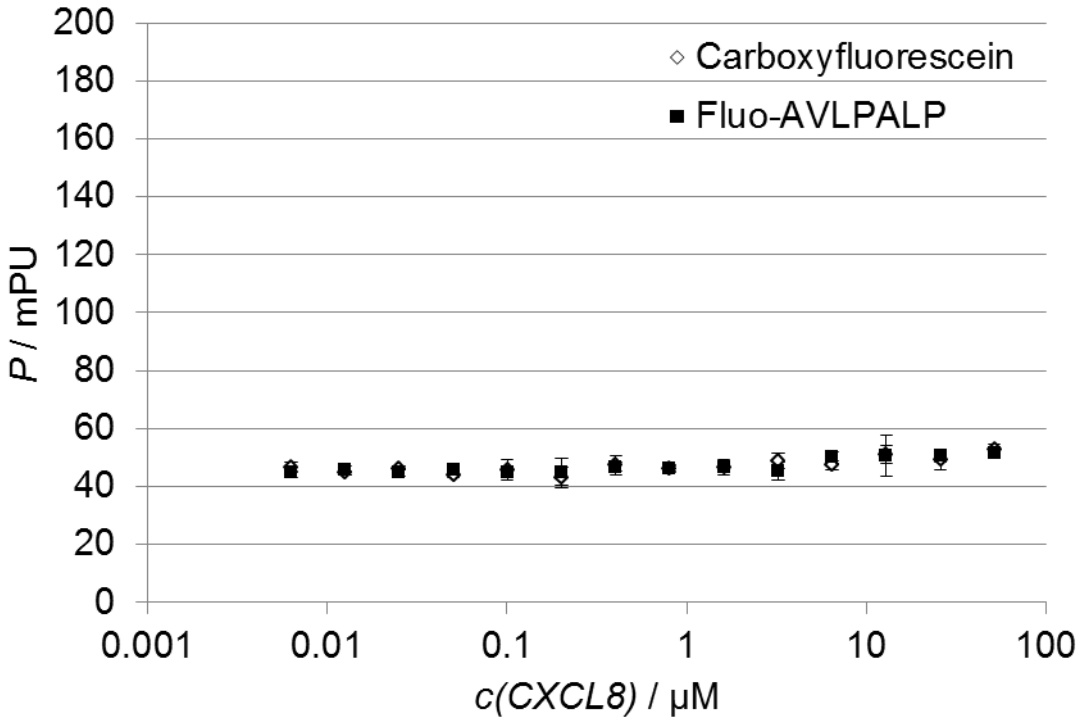

Figure S2: Controls for non-specific binding. Interactions of CXCL8 with carboxyfluorescein (white diamonds) and an unrelated fluorescently labeled peptide AVLPALP (black squares) both 20 nM in buffer C supplemented with 0,1% Triton X-100. No significant interactions can be detected for the fluorophor alone or the labeled peptide. Error bars indicate standard deviation of 3 measurements.

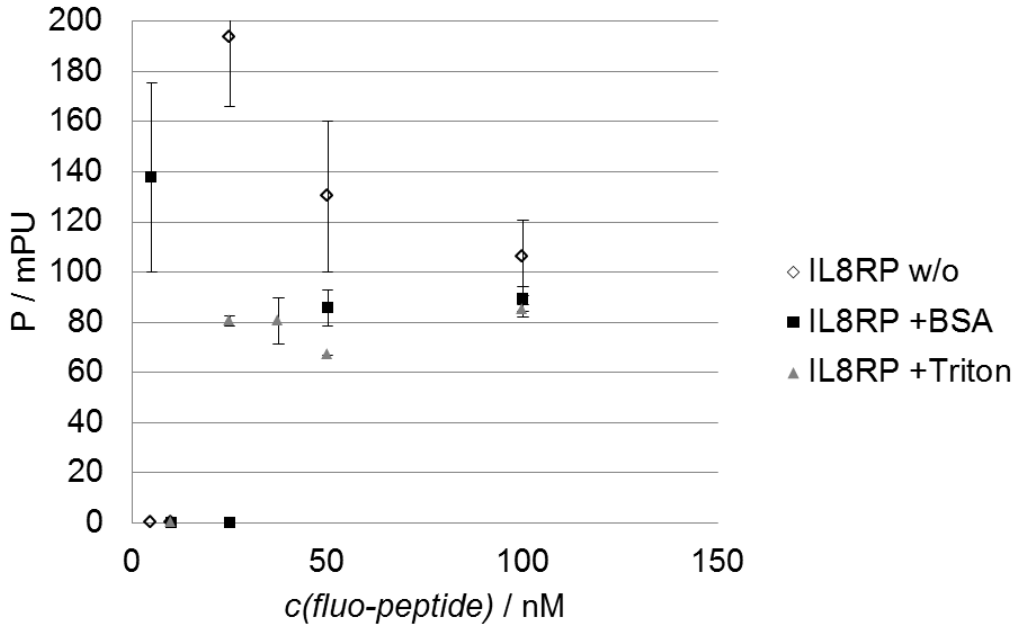

Figure S3: Influence of buffer supplements on fluorescence polarization of free fluorescein-labeled Ahx-receptor peptide in buffer A. (white diamonds) no supplements, (black squares) addition of BSA, (grey triangles) addition of Triton X-100. Increased values at low peptide

concentration indicate non-specific interactions with either vessel walls or added protein. Measurements marked as invalid by the instrument due to low intensity are indicated as “zero” values. Error bars indicate standard deviation of 3 replicates.

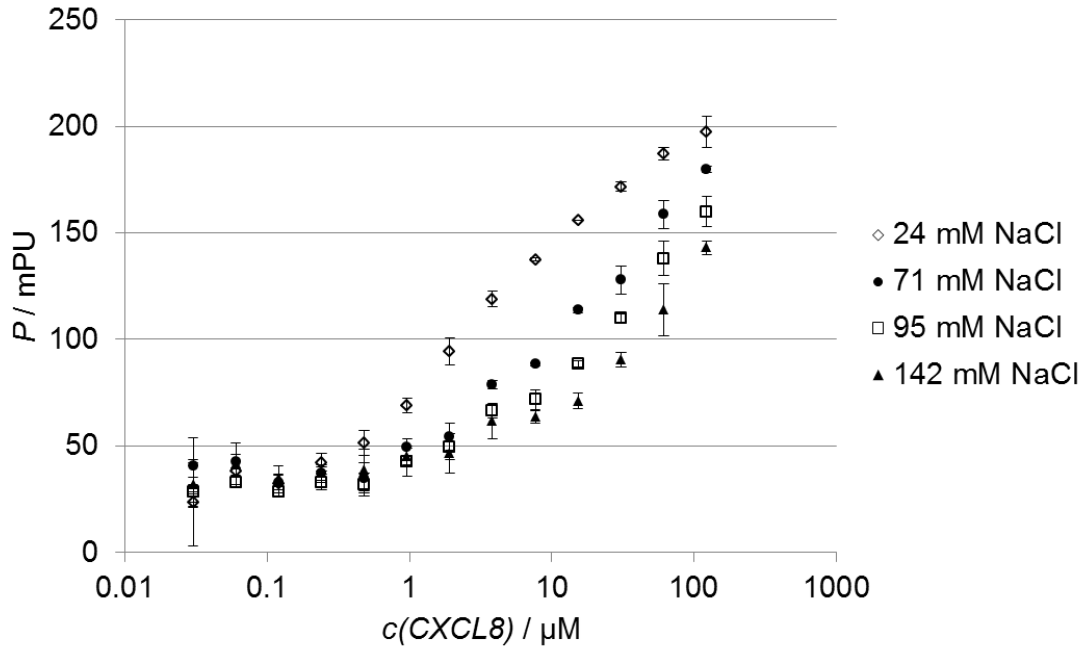

Figure S4: Titration experiments in the range of 24 to 142 mM sodium chloride. At the lowest concentration of 24 mM NaCl in 10 mM phosphate buffer, the K_d was only 3.4 ± 0.4 µM as compared to 61 ± 16 µM at 142 mM NaCl.

Table S1: Comparison of peptides derived from CXCR1 and conditions under which interaction with CXCL8 was studied

Publication	**Peptide-Sequence**	**Origin**	**Buffer**
NCBI NP_000625.1	MSNITDPQMWDFDDLNFTGMPPADEDYSPCMLETETLNK	human CXCR1 (1-39)	n.a.
NCBI NP_001164553.1	DLWTWFEDEFANATGMPPVEKDYSPCLVVTQTLNK	rabbit CXCR1 (10-43)	n.a.
Ravindran, 2009	LWTWFEDEFANATGMPPVEKDYSP	rabbit CXCR1 (11-34)	50 mM NaAc; **pH 6.0**; 1 mM 2,2-dimethyl-2-silapentane sulfonic acid, 1 mM sodium azide
Rajagopalan, 2004	LWTWFEDEFANATGMPPVEKDYSP LWTWFEDEFANATGMPPVEKDYSPCLVVTQTLNK	rabbit CXCR1 (11-34) rabbit CXCR1 (11-43)	in micelles; 50 mM Tris (**pH 8.0**), 50 mM NaCl
Attwood, 1997	ac-MWDFDD-Ahx-MPPADEDYSP-nh2 ac-MWDFDD----GMPPADEDYSP-nh2	human CXCR1 (9-14/Ahx/20-29) human CXCR1 (9-14/19-29)	PBS with 1% BSA, 0.1% NaN_3 (**pH 7,4?**)
Skelton, 1999	ac-MWDFDD-Ahx-MPPADEDYSP-nh2	human CXCR1 (9-14/Ahx/20-29)	50 mM phosphate, **pH 5.5**; 10% D_2O
Clubb, 1994	MSNITDPQMWDFDDLNFTGMPPADEDYSPSMLETETLNKY	human CXCR1 (1-40) C30S	50 mM potassium phosphate, **pH 6.7**
Leong, 1994	MSNITDPQMWDFDDLNFTGMPPADEDYSPCMLETETLNK	human CXCR1 (1-39)	25 mM HEPES, Hanks Minimal Medium, **pH 7.2**