Supplementary Material

Deciphering the *In-vitro* Homo and Hetero Oligomerization Characteristics of CXCL1/CXCL2 Chemokines

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Experimental Methods

Cloning, expression and purification of murine CXCL1 and CXCL2

Murine CXCL1 and CXCL2 genes were cloned in a customized pET32 vector as thioredoxin (Thx) fusion proteins along with a TEV cleavage site (Thx-TEV-CXCL1/2). Plasmids encoding for the corresponding genes were transformed in E.coli BL21(DE3) competent cells. Transformed cells were used to grow overnight seed culture (10 ml), and transferred to 1L large culture of LB medium or isotopically enriched $^{15}N/^{13}C$ minimal medium containing $^{15}NH_4Cl/^{13}C$-glucose as a sole source of nitrogen and carbon in the presence of 100µg/ml ampicillin at 37°C, 220 rpm until its OD reaches 0.6 at 600 nm. Proteins were expressed by inducing the cultures with 0.2 mM isopropyl 1-thio-β-D galactopyranoside (IPTG) and were grown at 20°C for 20 hours. Cells were harvested by centrifugation and the cell pellet was stored at -80°C. Cells were thawed, resuspended in lysis buffer (50mM Tris, 500mM NaCl pH 8), treated with lysozyme (100µg/ml) on ice for 1 hour and were lysed by sonication. Cell lysates were centrifuged at 14000rpm for 60 minutes at 4°C and supernatant was separated. Proteins (mCXCL1 and mCXCL2) found in cytoplasmic fraction (supernatant) were then purified using Ni-NTA column pre-equilibrated with lysis buffer (20mM Tris, 500mM NaCl pH 8). Proteins were eluted in the same lysis buffer using 400mM imidazole. Eluted proteins were dialyzed against a buffer containing 20mM Tris (pH 8), 50mM NaCl, 1mM BME and 0.2mM EDTA. After dialysis, Thx fusion protein was removed at TEV site through TEV digestion for 12-14 hours at 25°C. Proteins from the digested sample were separated via another round of Ni-NTA purifications. Proteins of interest (mCXCL1, mCXCL2) were collected from flow through,

were dialyzed and passed through S-column pre-equilibrated with buffer containing 20mM tris at pH7 and 50mM NaCl. Proteins were eluted with 250-500 mM NaCl and further purified using Superdex-75 Gel filtration column. Purity of the proteins was assessed using 15% SDS PAGE.

Glutaraldehyde Cross linking Assay

Glutaraldehyde cross linking assay[1] was performed by preparing dilutions (1%, 0.5%, 0.1%, 0.05%) of 25% glutaraldehyde (sigma) in Milli Q water. 1mg/ml protein samples of mCXCL1 and mCXCL2 in 100mM sodium phosphate buffer incubated with different concentration (0.0005%, 0.001%, 0.005%, 0.01%) of glutaraldehyde at 25 ^{0}C for 24 hours for cross linking. Reaction was stopped by adding SDS sample loading buffer. All the samples were analyzed using 15% SDS PAGE. Intensities of monomer, dimer and tetramer peaks observed for mCXCL1 and mCXCL2 were calculated using Image J software[2].

Contact Map

A comparative contact map depicting Cα contacts in mCXCL1 and mCXCL2 has been generated using contact map view (CM view) software[3] with distance threshold of 6 Å. 3D coordinates for mCXCL1 and CXCL2 were taken using the NMR data as described elsewhere[4,5].

NMR Data Acquisition and Processing

NMR experiments were carried out using a triple channel Bruker 500 MHz spectrometer equipped with a TXI cryoprobe. For ^{1}H-^{15}N HSQC and triple resonance experiments ^{15}N labeled mCXCL1/CXCL2 proteins were prepared in 50 mM sodium phosphate buffer (pH 6.0, 10% D_2O, 25^{o}C). Hetero dimer formation of mCXCL1-mCXCL2 was monitored by mixing ^{15}N-mCXCL1 (60 μM) with unlabeled-mCXCL2 in the ratio of 1:1. HSQC experiments were recorded with 128 scans and 128 complex increments. Spectra were processed and analyzed with NMRPipe[6], Bruker Topspin 3.2 and NMR View[7].

Generation of homo/hetero-oligomeric structures

Structures for mCXCL2 (Pdb ID: 1MI2, 3N52) was available in RCSB protein data bank. Structural model for mCXCL3 was generated through homology modeling based on target template alignment using Promod II in Swiss model server[8] by employing **3N52** PDB as

template[9], and mCXCL1 structure was modeled using NMR data as described elsewhere[4]. Structural models for both mCXCL1 and mCXCL3 were energy minimized to reduce the side chain/backbone abnormalities and their quality was validated using PROCHECK and visualized using Ramachandran statistics[10]. Heterodimers were generated through the monomeric counterparts of mCXCL1/mCXCL2/mCXCL3 structures by performing symmetry operations employing Pymol macros using Cα chains and dimer interface residues as reference restraints. Surfaces for homo/hetero-dimeric mCXCL1-3 chemokines were generated exploiting surface generation in Pymol molecular graphic system[11].

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Sfig. 1: Overlay of ^{1}H-^{15}N HSQC spectra of ^{15}N-mCXCL1 (blue) and ^{15}N-mCXCL1+^{14}N-mCXCL2 (red) in the ratio of 1:1 showing the interaction of mCXCL1 with mCXCL2. Some of the well resolved resonances appeared in the complex spectra upon formation of the heterodimer are encircled in green.