

## **Abstract**

CD69 is considered as the marker receptor of activated lymphocytes and is expressed at sites of active immune response. Physiologically it appears in the form of covalently bound homodimer, however after examining its three-dimensional structure we suggested Q93 on one subunit and D88 with E87 on the other one to participate on the inter-subunit interactions. Even more profound intertwining was observed in case of R134 of one subunit with A136 and Y135 on the other one. Therefore Q93, R134 or both were mutated into alanines and showed the monomeric form just in case of double-mutant. This fact influenced significantly also the binding of ligands. While the  $K_d$  values for binding of GlcNAc was approximately  $10^{-5}$  M in case of monomeric form, in case of dimeric form it was 10 times lower and even 100 times lower in case of the longest covalently bound dimers. Although the gel filtration retention time decrease was observed, which could indicate a change in molecular fold, the value of experimentally determined sedimentation coefficient was identical. Moreover neither the comparison of HSQC NMR spectra before and after ligand saturation revealed any significant shifts.

Hydrogen deuterium exchange is a chemical process in which a covalently bonded hydrogen atom is replaced by deuterium or vice versa. As the automatic searching for peptides and determination of its exact monoisotopic mass from mass spectra is based on natural abundance of particular isotopes and/or virtual average amino acid average and subsequent calculated shape of isotopic envelope, embedded automatic peptide searching tools in common MS data processing software applications are not able to assign the envelope deformed and shifted by unnatural addition of deuteriums to be a peptide. Therefore we developed the macro for DataAnalysis (Bruker Daltonics) application and a software suite for HXMS data visualization.

## **Keywords**

CD69, CD69 structure, ligand binding, H/D exchange, automatic MS data evaluation, H/D exchange data visualization