# Impact of Tetramerization on Neuraminidase Dynamics and Binding Site Conformations

*Susanne von Grafenstein[1], Hannes G. Wallnoefer[1], Julian E. Fuchs[1], Roland G. Huber[1], Judith M. Rollinger[2], Johannes Kirchmair[3], Klaus R. Liedl[1]*

*[1]Institute of General, Inorganic and Theoretical Chemistry and Center for Molecular Biosciences, University of Innsbruck*
*Innrain 52a, Innsbruck, Austria*
*[2]Institut of Pharmacy and Center for Molecular Biosciences, University of Innsbruck*
*Innrain 52c, Innsbruck, Austria*
*[3]Unilever Centre for Molecular Informatics, University of Cambridge*
*Lensfield Road, Cambridge, United Kingdom*

Influenza neuraminidase is a tetrameric surface protein of the influenza virus and the target for antiviral drugs e.g., oseltamivir and zanamivir [1]. The conformational diversity of the 150-loop was revealed by crystal structures of the group 1 neuraminidases [2] and investigated by molecular dynamics (MD) simulations [3], [4]. The open state conformation shows an additional sub-pocket (150-cavity) exploitable for drug design [1], [5], [6].

We compared tetrameric influenza neuraminidase with monomeric neuraminidases of other organisms and identified a unique insertion typical for the influenza variant. These residues are part of the protein-protein-interface as well as the flexible 150-loop. We assume this insertion to mediate the tetramerization effects via dynamics.

Therefore, we present a systematic analysis of three neuraminidases (avian 2005, pandemic 1918, pandemic 2009) with all-atom, explicit solvent MD simulations applying the Amber forcefield ff99SB. Comparative simulations of monomeric, dimeric and tetrameric systems show, that the sampled conformational phase space for tetramer is distinct from monomer simulations. We demonstrate, that interactions with adjacent neuraminidase subunits alter the dynamics of the 150-loop.

These results underline the importance of protein-protein-interactions in the influenza neuraminidase tetramer for the examination of molecular flexibility, especially for the loop forming the 150-cavity. In consequence, considering these interactions is crucial for drug development.

[1] U. Grienke, M. Schmidtke, S. von Grafenstein, J. Kirchmair , K. R. Liedl, J. M. Rollinger, *Nat Prod Rep*, **2012,** *29*, 11-36
[2] R.J. Russell, L.F. Haire, D.J. Stevens, P.J. Collins, Y.P. Lin., G.M. Blackburn, A.J Hay, S.J. Gamblin, J.J. Skehel. *Nature*, **2006,** *443*, 45-49.
[3] R. E. Amaro, D. D. L. Minh, L. S. Cheng, W.M. Lindstrom, A.J. Olson, J.H Lin, W.W. Li, J.A. McCammon, *J Am Chem Soc*, **2007,** *129*, 7764-7765.
[4] R.E. Amaro, X.L. Cheng, I. Ivanov, D. Xu, J.A. McCammon, *J Am Chem Soc*, **2009,** *131*, 4702-4709.
[5] U. Grienke, M. Schmidtke, J. Kirchmair, K. Pfarr, P. Wutzler, R. Durrwald, G. Wolber, K.R. Liedl, H. Stuppner, J.M. Rollinger, *J Med Chem*, **2010**, *53*, 778-786.
[6] S. Rudrawar, J. C. Dyason, M.-A. Rameix-Welti, F.J. Rose, P.S. Kerry, R.J.M. Russell, S. van der Werf, R.J. Thomson, N. Naffakh, M. von Itzstein, *Nat Commun*, **2010,** *1*, 113