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Biophysical studies of influenza virus proteins involved in membrane fusion and host RNA cleavage

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Membrane fusion induced by hemagglutinin fragment, the so-called fusion peptide (HAfp), and host RNA cleavage by viral polymerase are the key steps of influenza replication. A 20-amino acid HAfp1-20 peptide has a boomerang-like shape, however it has been shown recently that extending HAfp1-20 by three more conservative residues (to HAfp1-23) leads to a helical hairpin formation. We determined partition coefficients K_x for a series of peptides in their native forms using tryptophan fluorescence. HAfp1-23 showed more favorable interaction than HAfp1-20 with DOPC at pH 7.4, but at endosomal pH 5.0 the difference was negligible. Both peptides lead to liposome content leakage in a similar fashion, as measured in single giant unilamellar vesicles (GUV) using fluorescence microscopy. Nevertheless HAfp1-23 had larger liposome fusion capacity, as concluded from FRET experiments and showed a distinct lipid bilayer distortion by fluorescence lifetime imaging. Despite intensive studies on endonucleolytic polymerase domain (PA-Nter), the existing results on divalent ion preference are contradictory. We quantified the PA-Nter cleavage reaction rates by fluorescence cross-correlation spectroscopy (FCCS). This microscopic technique provides rapid, highly sensitive and real-time monitoring of single molecule interactions. In the regime of enzyme excess, using ss-DNA at nanomolar concentrations, we determined the maximum reaction rates at 0.51 and 0.77 nM/min for Mg^{2+} and Mn^{2+} , respectively. Our results show the superiority of FCCS technique for real-time kinetic analysis over the electrophoretic assays. Presented studies constitute a step towards better understanding of fusion and RNA cleavage mechanisms.

Biography

Remigiusz Worch has completed his PhD at the University of Warsaw in 2007 and Post-doctoral studies at the Biotechnology Center of the Technical University Dresden, Germany (BIOTEC) as the Alexander von Humboldt fellow. Currently he is an Assistant Professor at the Biological Physics Group, Institute of Physics, Polish Academy of Sciences in Warsaw, Poland.

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