CryoEM Structure of Dynamin-like MxB in Assembly

Peijun Zhang[1,2,3,4], Frances Joan D. Alvarez[3,4], Shaoda He[5], Juan R. Perilla[6], Sooin Jang[4,7,8], Alan N. Engelman[4,7,8], Sjors H.W. Scheres[5]

[1]Division of Structural Biology, University of Oxford, The Henry Wellcome Building for Genomic Medicine, Headington, Oxford OX3 7BN, UK
[2]Electron Bio-Imaging Centre, Diamond Light Sources, Harwell Science and Innovation Campus, Didcot OX11 0DE, UK
[3]Department of Structural Biology, [4]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260, USA
[5]MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge CB2 0QH, UK
[6]Department of Physics and Beckman Institute, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA
[7]Department of Cancer Immunology and Virology, Dana-Farber Cancer Institute, Boston, MA 02215, USA
[8]Department of Medicine, Harvard Medical School, Boston, MA 02215, USA

Human dynamin-like, interferon-induced myxovirus resistance 2 (Mx2/MxB) is a potent inhibitor of HIV-1 infection and a potential agent for the treatment of HIV/AIDS. MxB directly interacts with the HIV-1 capsid and blocks nuclear import of pre-integration complexes and subsequent chromosomal integration of the viral cDNA. Anti-HIV-1 activity and capsid-binding require the N-terminal domain of MxB and protein oligomerization, yet each of these has eluded structural determination due to difficulties in protein preparation. We purified a GTPase active MBP fusion of the full-length wild-type MxB from mammalian cells. The full-length MxB purifies as discrete oligomers and further self-assembles into helical arrays in physiological salt. Intriguingly, GTP, but not GDP, binding to MxB results in array disassembly, while subsequent GTP hydrolysis allows its re-formation. Using cryoEM, we determined the MxB assembly structure at 4.6 Å resolution, revealing novel oligomerization and higher-order assembly interfaces that were absent or are distinct from the crystal structure of MxB that lacked the N-terminal domain and harbored interface mutations. The structure suggests that salt bridges mediate MxB higher-order assembly, which is disrupted by GTP-induced conformational changes. More importantly, mutational analysis combined with viral infectivity assays revealed that MxB oligomers, not the dimer or higher-order assemblies, are in fact the active species against HIV-1 infection. The near-atomic wild-type MxB structure provides a vital framework for re-evaluating conflicting results regarding the effects of MxB oligomerization and GTPase activity on its anti-HIV-1 activity. Moreover, this first high-resolution assembly structure among the superfamily of dynamin-like large GTPases allows us to propose a new GTP-dependent assembly/disassembly model, distinct from current models.