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Investigation of Structural and Functional Relationship of Vaccinia Virus Envelope Protein A26 by NMR and SPR Spectroscopy

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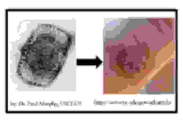
Abstract

Vaccinia virus (Vacv) is a prototype poxvirus that infects cells via endocytosis and plasma membrane fusion. It is the active constituent of the vaccine that eradicated smallpox. With promising applications for gene therapy and genetic engineering as well as with threats of smallpox being used for bioterrorism, scientists have continuously studied the Vacv structure as well as its virus cell entry mechanism.

Virus entry into host cells relies on lipid, carbohydrates or protein interactions between the cell and virus. Protein-protein interaction, particularly is said to play a pivotal role in coordinating the virus binding and uptake. Two envelope proteins that play critical role in Vacv cell entry are the pH sensitive A26 proteins (500 a.a, regulates virus entry pathway) (Chang, E., et al., *J. Virol.* 84, 8422-8432 (2010) and the 110 a.a. long A27 protein that acts as mediator between A26 and the transmembrane protein A17 (Chiu, et.al., *J. Virol.* 85, 2149-2157 (2007). Vacv protein A26 interacts with A27 for localization in the mature virion surface through the transmembrane protein A17 directly attached to the A27 while binding with extracellular matrix laminin for virus attachment to the host cell. In this study we probed the interactions between A26-A27 and A26-laminin using solution NMR.

Through 2D and 3D NMR analysis paired with Surface Plasmon resonance and protease mapping using MALDI-TOF, we were able to predict the C terminal structures of A26 and A27 respectively, as well as identify overlapping binding sites involved in the A27-A26-laminin interactions. A26 C-terminal charged amino acids (420-500 a.a.) interact with A27 (70-110 aa) while hydrophobic amino acids (400-500 a.a.) bind with the α5LG4 site of laminin. A27 on the other hand uses its leucine zipper like domain to bind both with A26. All interactions showed comparable binding strength in the 10^{-7} M range. Competition experiments and point mutations are all being done to help identify how each interactions behave during virus entry into the host cell.

I. INTRODUCTION AND METHODOLOGY



Vaccinia Virus (VACV)

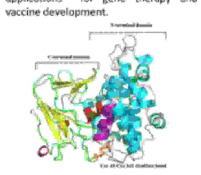
Vacv is a large, complex virus from the Poxvirus Family with promising applications for gene therapy and vaccine development.

Scientific Problem

A26 (laminin binding), A27 (heparan sulfate binding) are vacv proteins important for virus cell entry.

In vivo studies have not yet identified the structure and specific interactions involved in the A27-A26-laminin complex formation.

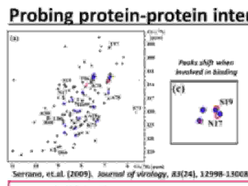
Ching, Y.-C., et al., *J. Virol.* 83, 6404-16 (2009)
Howard, A.R., et al., *J. Virol.* 82, 12361-12391 (2008)
Ching, E., et al., *J. Virol.* 84, 8422-8432 (2010)



Only the A26 17-364 region visible in the X-ray crystal

Chang, et.al. Plos Pathogens (2015) – Recently Accepted manuscript

Probing protein-protein interactions with NMR



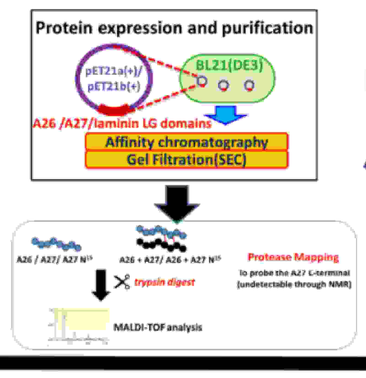
Nuclear Magnetic Resonance (NMR) can probe protein interactions at atomic resolution.

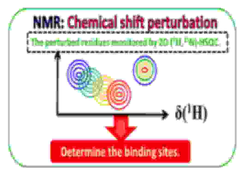
We therefore propose to apply NMR for a more in-depth analysis of the A27-A26 protein-protein interaction as well as to the A26-laminin binding

Serrano, et.al. (2009). *Journal of virology*, *83*(24), 12998-13008.

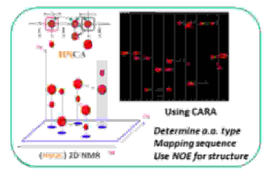
Research Objectives:

1. Studying A26-A27 and A26-laminin interactions *in vitro* and identifying the active binding sites by NMR & Mass spectrometry
2. Establish molecular models of A27/A26 and A26/laminin complex formation to elucidate its underlying binding mechanism

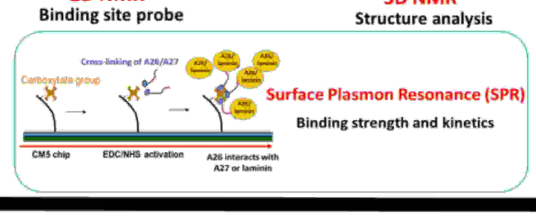




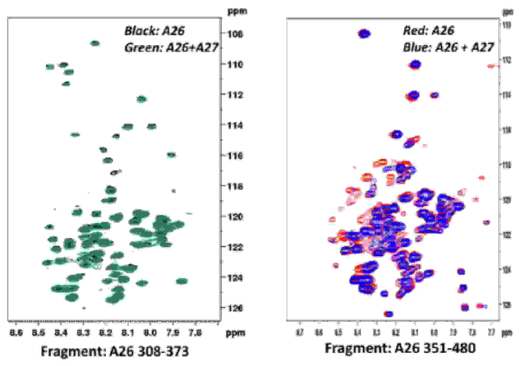
2D NMR
Binding site probe



3D NMR
Structure analysis



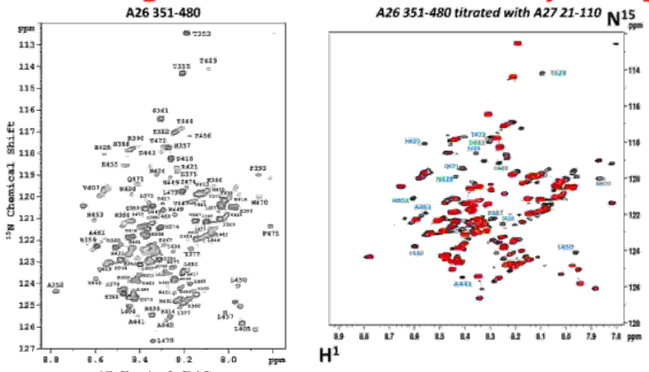
II. A26 binding with A27 - *A26 binds A27 using 425-472 a.a. with mostly charged and polar residues.*



Fragment: A26 308-373 | Fragment: A26 351-480

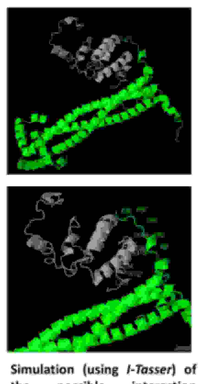
A26 C-terminal fragment 351 – 480 a.a binds with A27 at pH 5.0

Wang, Da-Rong, et al Journal of Biological Chemistry(2014): jbc-M114.



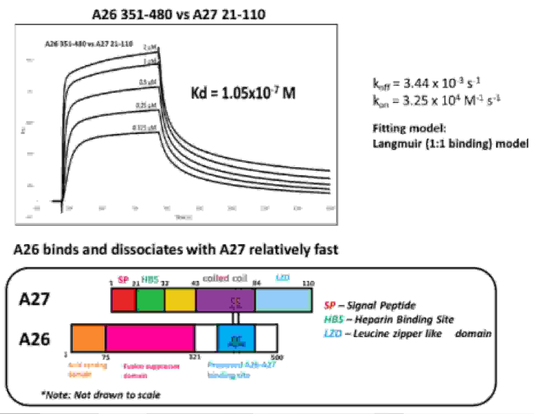
3D NMR analysis of A26 C-terminal was done. After sequencing, NMR titration was conducted and specific amino acids involved in binding were identified.

Wishart, David S., et, al. *Biochemistry* 31.6 (1992): 1647-1651.

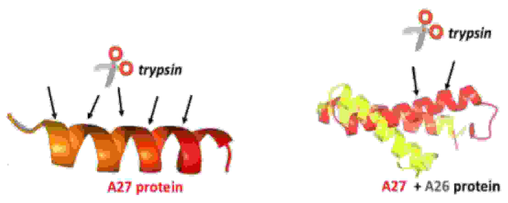


Simulation (using *I-Tasser*) of the possible interaction between A27 21-110 (green) and A26 351-480 (grey)

SPR data:



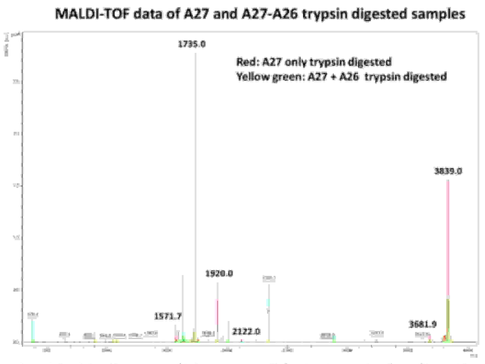
III. A27 binding with A26 - *A27 binds A26 using its LZD region.*



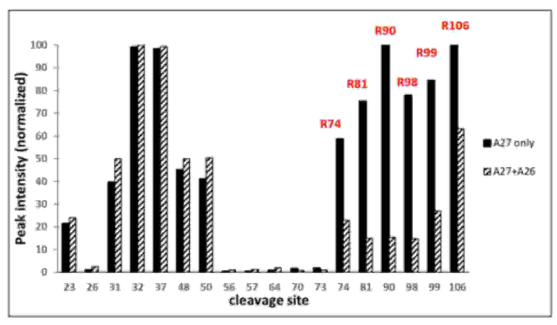
Protease mapping **was applied for the NMR invisible C-terminal of A27 to identify the protein's A26 binding site.**

Kriwacki, R. W., et.al(1996). *Journal of the American Chemical Society*, *118*(22), 5320-5321.

MALDI-TOF data of A27 and A27-A26 trypsin digested samples

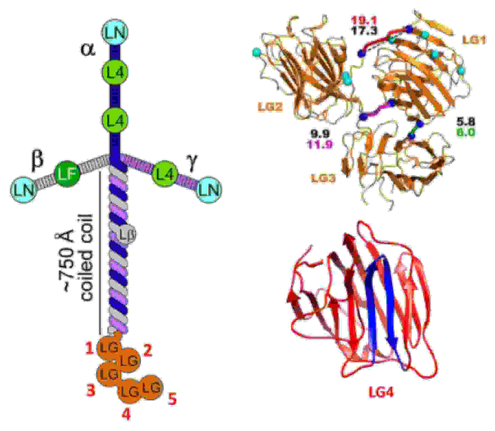


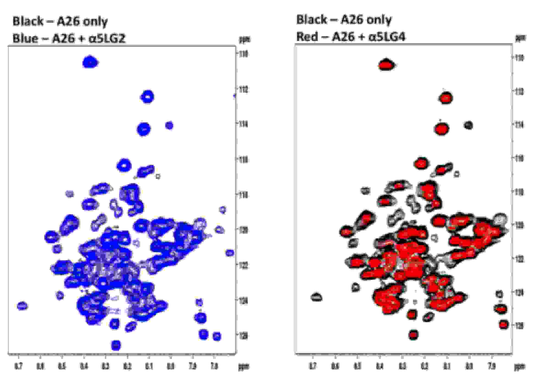
A27 site involved in binding won't be accessible to trypsin for digestion leading to a decrease or loss of the fragment peak in the MALDI-TOF analysis.



Results show that A27 binds A26 through its leucine zipper like domain.

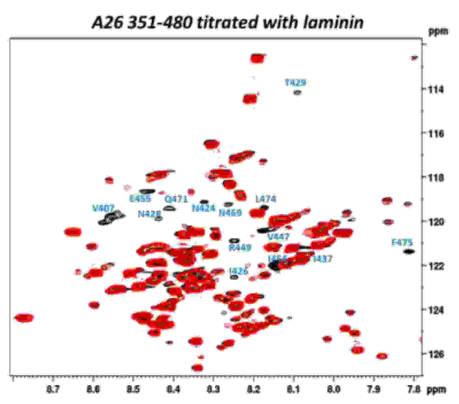
IV. A26 binding with laminin - *A26 binds α5LG4 domain of laminin using 407-475 a.a. with mostly hydrophobic residues*





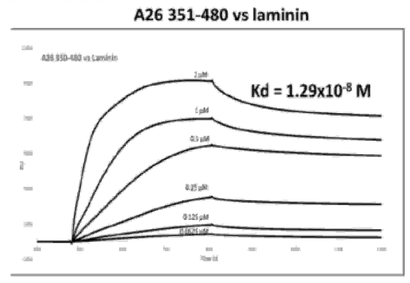
Results show α5LG4 domain of laminin binds with A26.

Armony, G., et.al. (2016). *Proceedings of the National Academy of Sciences*, *113*(47), 13384-13389.



Results show α5LG4 region binds with A26 407-475 aa perturbing mostly *hydrophobic residues.*

SPR data:



A26 binds and dissociates with laminin relatively slow

Acknowledgements
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