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## Characterization of the entry mechanism of a novel protein transduction domain originated from Betanodavirus

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**B**etanodavirus, also called nervous necrosis virus (NNV), is the causative agent of a fish disease viral nervous necrosis. VNN causes high mortality in marine fish at larvae and juvenile stages resulting in heavy financial losses of marine aquaculture industry. Base on the sequence analysis and 3D structure elucidation of orange-spotted grouper nervous necrosis virus (OGNNV), we found a peptide with the capsid protein can carry foreign protein to enter fish cells. Through cell entry experiment with different sections of the peptide fused with GFP, we characterized a novel 14-aa peptide as protein transduction domain (PTD) and named NNV-PTD. NNV-PTD can be fused with foreign protein at N- or C-terminal without disturbing the entry efficiency. In addition, NNV-PTD can enter different types of fish cells, insect cells and mammalian cells as well as enters fish cells with higher efficiency than well-known PTD such as TAT, Penetratin and R8. NNV-PTD entered fish cells to perinuclear regions within 4 h and can be blocked by culturing at 4, indicating the endocytosis-dependent entry mechanism. Therefore, we used the biochemical inhibitors blocking clathrin-

mediated endocytosis (CME), micropinocytosis, caveolae-dependent endocytosis, cellular cholesterol, low-pH balance and cytoskeleton to treat cells before entry assay. Finally, we demonstrated that NNV-PTD enters fish cells via CME depending on dynamin and macropinocytosis depending on myosin  $\alpha$ , and also the entry is cholesterol-, low-pH, and cytoskeleton-dependent at the intracellular traffic level. Together, this work not only characterizes a novel high efficient PTD but also identifies its entry mechanism, providing basic information for further application of NNV-PTD in aquaculture.

### Speaker Biography

Junfeng Xie has completed his PhD from Sun Yat-sen University, China. He is the Associate Professor of School of Lifesciences, Sun Yat-sen University, USA. He is focusing on the basic virology research of Betanodavirus and on the application study of antiviral agents.

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