Research of the month : December 2015

Preclinical Research

CHEMICAL BIOLOGY & DRUG DESIGN

Chem Biol Drug Des. 2015 Nov;86(5):1093-104.

A Peptide Inhibitor Derived from the Conserved Ectodomain Region of DENV Membrane (M) Protein with Activity Against Dengue Virus Infection

Chem Biol Drug Des 2015; **86:** 1093–1104
Research Article
Impact factor = 2.802

A Peptide Inhibitor Derived from the Conserved Ectodomain Region of DENV Membrane (M) Protein with Activity Against Dengue Virus Infection

Aussara Panya[1,2], Nunghathai Sawasdee[1], Mutita Junking[1], Chatchawan Srisawat[2], Kiattawee Choowongkomon[3] and Pa-thai Yenchitsomanus[1,*]

[1]Division of Molecular Medicine, Department of Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand
[2]Department of Biochemistry, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand
[3]Department of Biochemistry, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand
**Corresponding author: Pa-thai Yenchitsomanus, pathai.yen@mahidol.ac.th*

Dengue virus (DENV) infection is a public health problem worldwide; thus, the development of a vaccine and anti-DENV drugs is urgently needed. It has been observed that low levels of viremia in DENV-infected individuals are associated with mild disease outcomes; therefore, reduction of DENV load should offer therapeutic benefits. Disruption of protein–protein interactions on the surface of DENV by a peptide that mimics part of its structural protein may affect stability of the virion structure and inhibit viral entry into host cells. To test this hypothesis, we generated a novel peptide inhibitor that mimics the conserved ectodomain region of DENV membrane (M) protein, MLH40 peptide, for DENV inhibition assays. MLH40 inhibited all four serotypes of the virus (DENV1–4) at half maximal inhibition concentration of 24–31 μM. MLH40 at 100 μM blocked DENV2 attachment to cells by 80%. The inhibitory activity of MLH40 against DENV was consistently observed with different cell types, including Vero, A549, and Huh7 cells. Prediction of MLH40 binding by a molecular docking program indicated that its N-terminal loop may interact with DENV envelope (E) proteins and alter their dimer conformation. Thus, MLH40 may serve as a lead-peptide inhibitor for the development of an anti-DENV drug.

Key words: Dengue virus, infection, molecular mimicry, peptide inhibitor, public health

Received 24 December 2014, revised 3 April 2015 and accepted for publication 15 April 2015

Dengue virus (DENV) infection is a mosquito-borne disease causing a spectrum of clinical symptoms ranging from mild febrile illness of dengue fever to more severe systemic disorders such as dengue hemorrhagic fever and dengue shock syndrome (1). DENV infection has emerged as a public health problem worldwide with over 2.5 billion people (> 40%) of the world population at risk and with an annual rate of 50–100 million people in more than 100 endemic countries infected with DENV (2). DENV belongs to the *Flavivirus* genus and *Flaviviridae* family comprising four antigenically distinct serotypes, DENV1–4. DENV infection normally generates low immunogenic cross-protection; thus, the infected persons can be infected later with different DENV serotypes. Intriguingly, subsequent infection with different DENV serotypes may cause more severe disease because non-neutralizing antibodies from earlier DENV infections augment the severity of later infection via the binding of the virus–antibody complex to Fc receptor-bearing cells. This enhances DENV entry into host cells, a mechanism termed the antibody-dependent enhancement phenomenon (3). This mechanism increases the challenge for the development of a safe and effective vaccine to provide protective immunity against all four DENV serotypes. Currently, DENV vaccine candidates are at the clinical trial stage (4,5). Although the development and availability of a DENV vaccine should provide effective DENV protection, an anti-DENV agent for therapeutic use in patients with active DENV infection is urgently required. It was previously reported that the levels of circulating DENV load in infected patients correlated with disease outcome and that low levels of viremia in DENV-infected individuals were associated with a mild form of DENV fever (6–9).

Peptides are natural molecules used by many living organisms to defend themselves from viral, bacterial, fungal, and parasitic infections (10). Peptides possess several characteristics suitable for the development of antiviral agents. First, they are generally safer or less toxic when used as drugs compared with synthetic compounds (11). Second, they are structurally and biochemically diverse, which allows for a rationale design to specific target proteins of interest and to elicit therapeutic responses (12). Third, there is an example of an approved peptide inhibitor, enfuvirtide, against human immunodeficiency virus (13),

 doi: 10.1111/cbdd.12576

A Peptide Inhibitor Derived from the Conserved Ectodomain Region of DENV Membrane (M) Protein with Activity Against Dengue Virus Infection

Prof. Dr. Pa-thai Yenchitsomanus
Department: Research and Development
Field of interests: Human Molecular Genetics, Human Genomics, Molecular Medicine, Cancer Immunotherapy
Contribution: Correspondent author

Aussara Panya
Department: Biochemistry, Research and Development
Contribution: First author

Nunghathai Sawasdee
Department: Research and Development
Contribution: Co-author

Mutita Junking
Department: Research and Development
Field of interests: Molecular Biology, Cell Biology
Contribution: Co-author

Asst. Prof. Chatchawan Srisawat
Department: Biochemistry
Field of interests: Biological Chemistry
Contribution: Co-author

A Peptide Inhibitor Derived from the Conserved Ectodomain Region of DENV Membrane (M) Protein with Activity Against Dengue Virus Infection

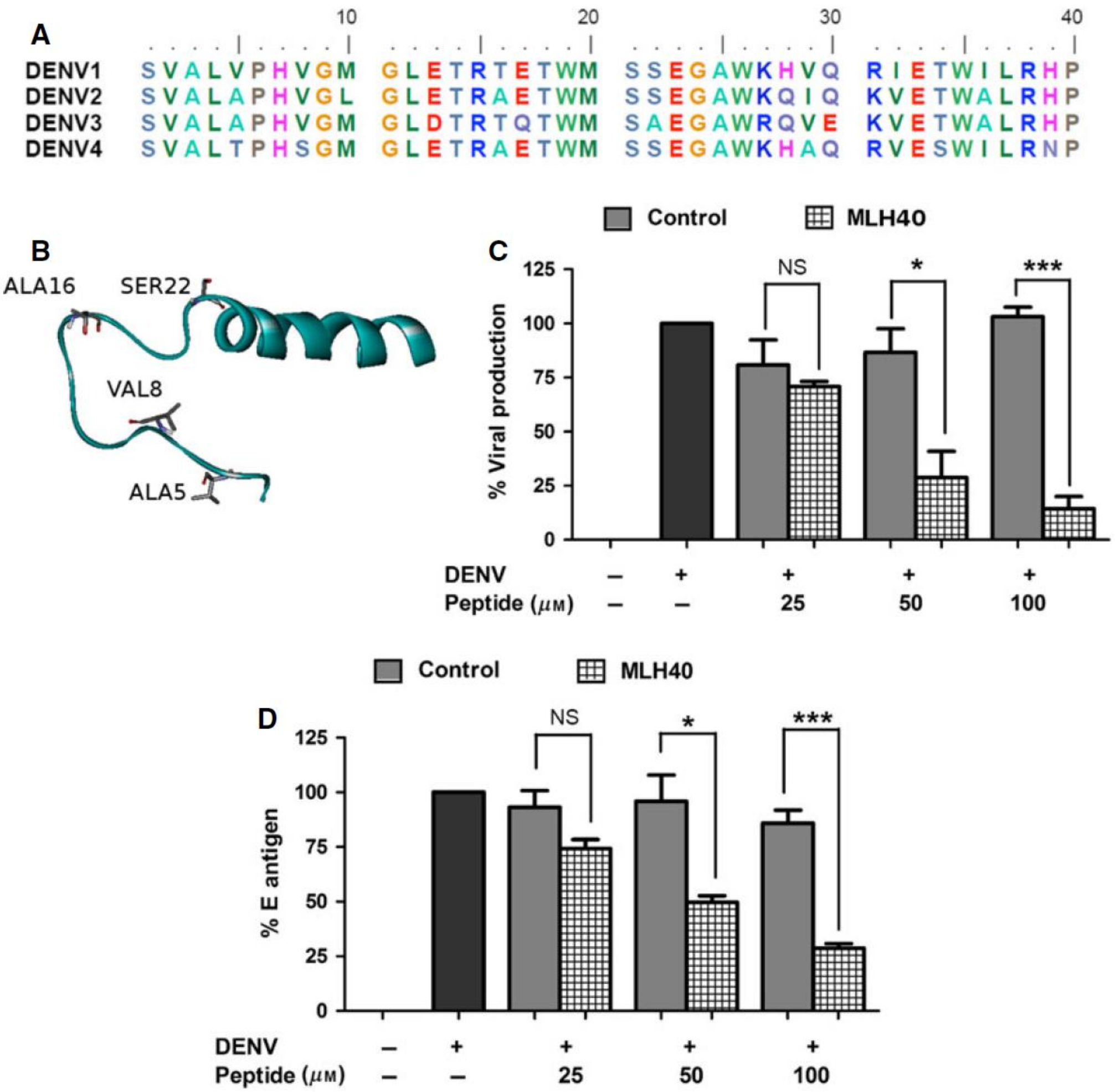

Figure 1: MLH40 peptide and its inhibitory effect on DENV infection in Vero cells.

A Peptide Inhibitor Derived from the Conserved Ectodomain Region of DENV Membrane (M) Protein with Activity Against Dengue Virus Infection

Panya et al.

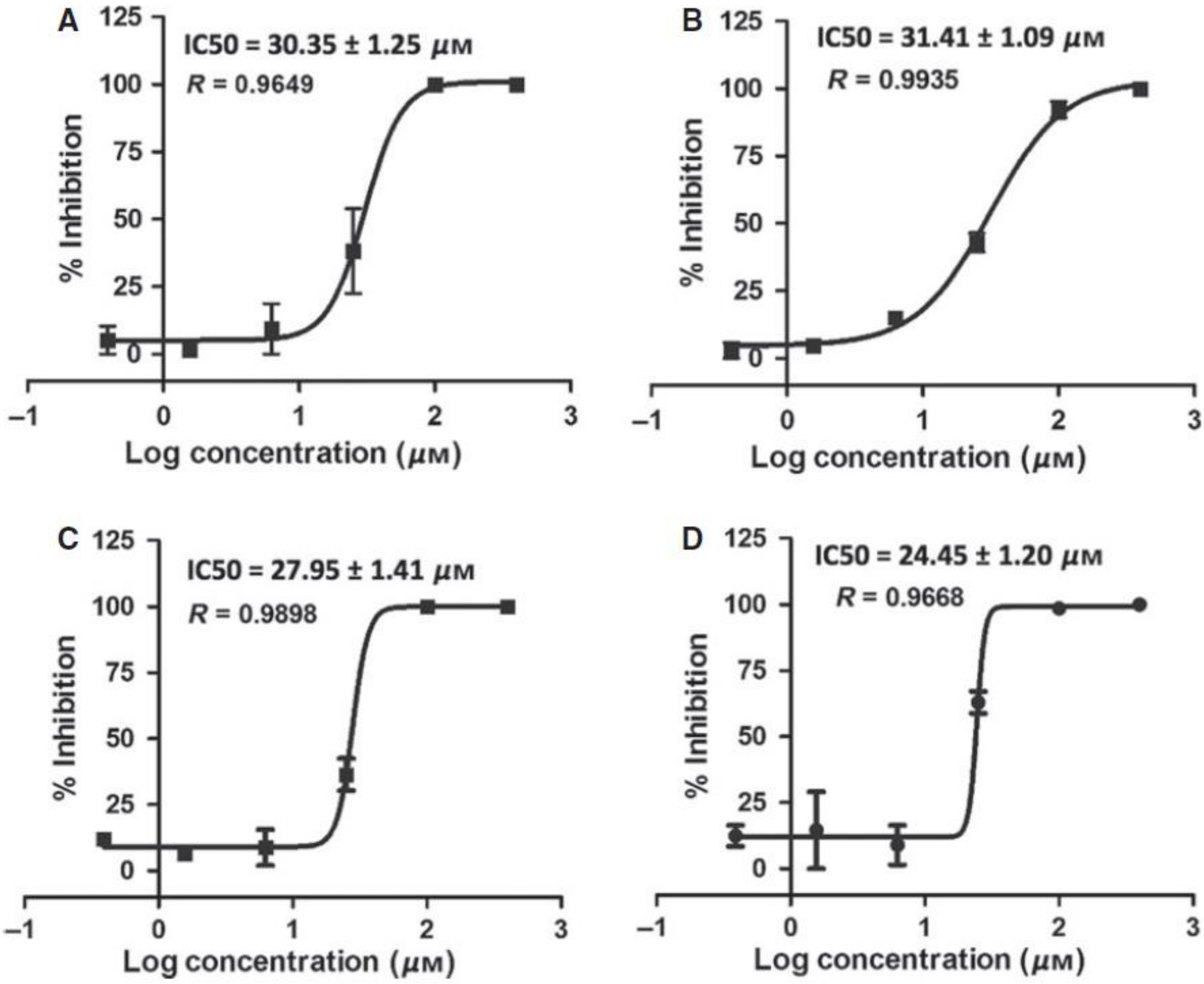

Figure 2: Half maximal inhibitory concentration (IC50) values of MLH40 peptide against four DENV serotypes.DENV1 (A), DENV2 (B), DENV3 (C), and DENV4 (D) were treated with MLH40 peptide

A Peptide Inhibitor Derived from the Conserved Ectodomain Region of DENV Membrane (M) Protein with Activity Against Dengue Virus Infection

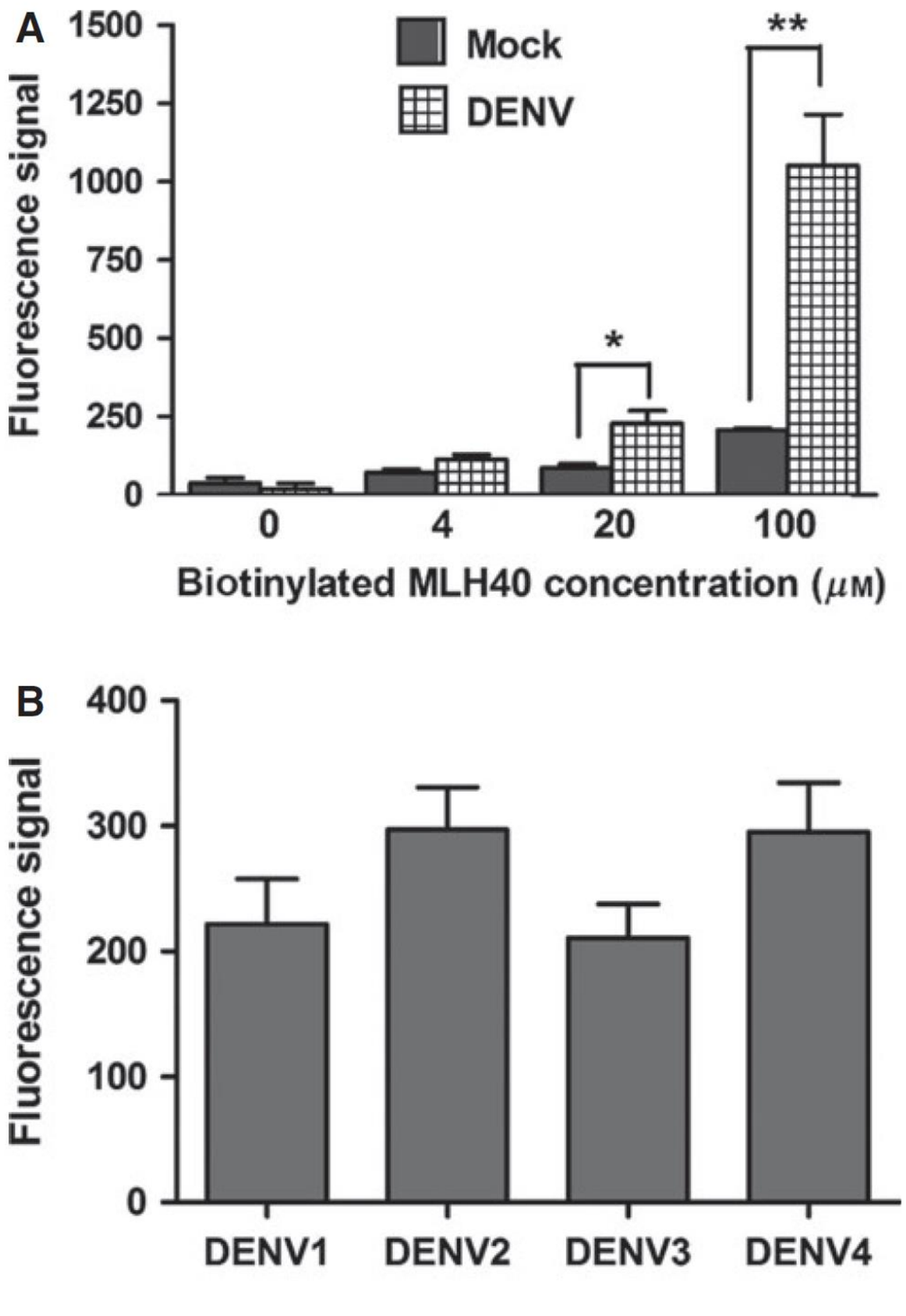

Figure 3: Binding of MLH40 peptide to DENV.

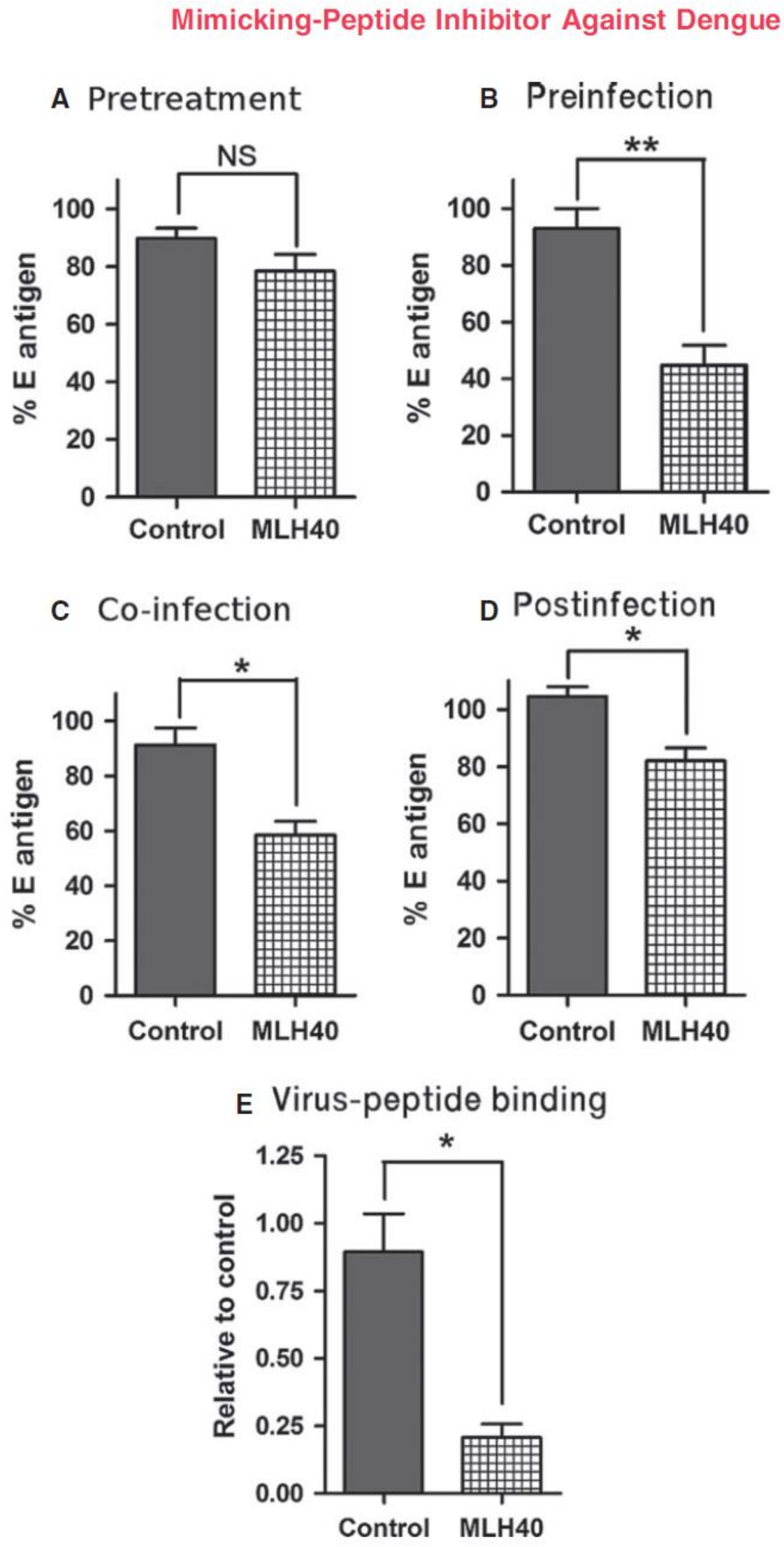

Figure 4: Effects of MLH40 peptide on time of addition and DENV2 infections. The inhibitory effect of 50 IM-MLH40 was tested to inhibit approximately 400 FFU of DENV2 based on the time of addition.

A Peptide Inhibitor Derived from the Conserved Ectodomain Region of DENV Membrane (M) Protein with Activity Against Dengue Virus Infection

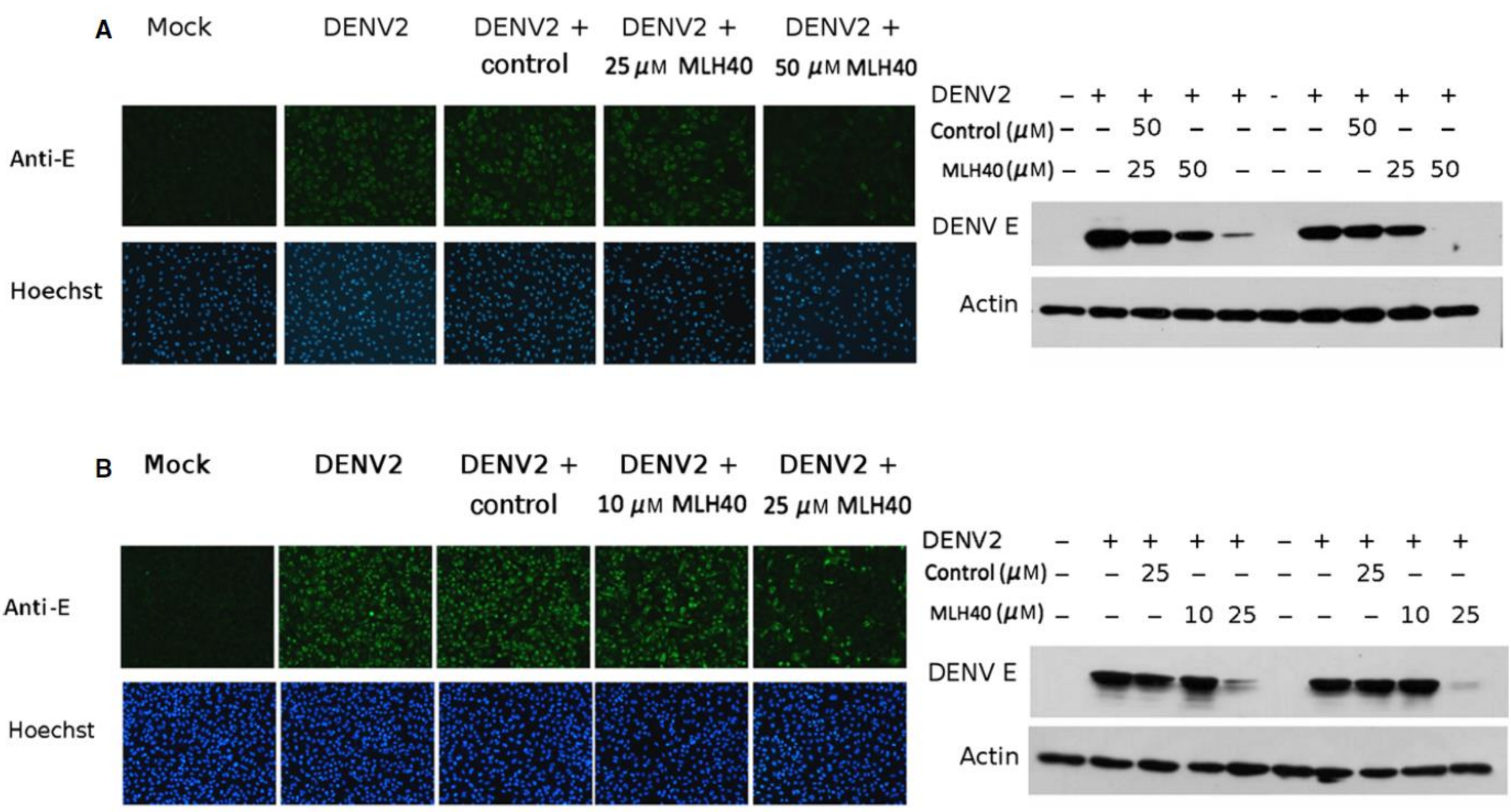

Figure 5: Inhibitory effects of MLH40 peptide on DENV2 in different cell types. DENV2 was treated with MLH40 before infection of Vero, A549, and Huh7 cells.

A Peptide Inhibitor Derived from the Conserved Ectodomain Region of DENV Membrane (M) Protein with Activity Against Dengue Virus Infection

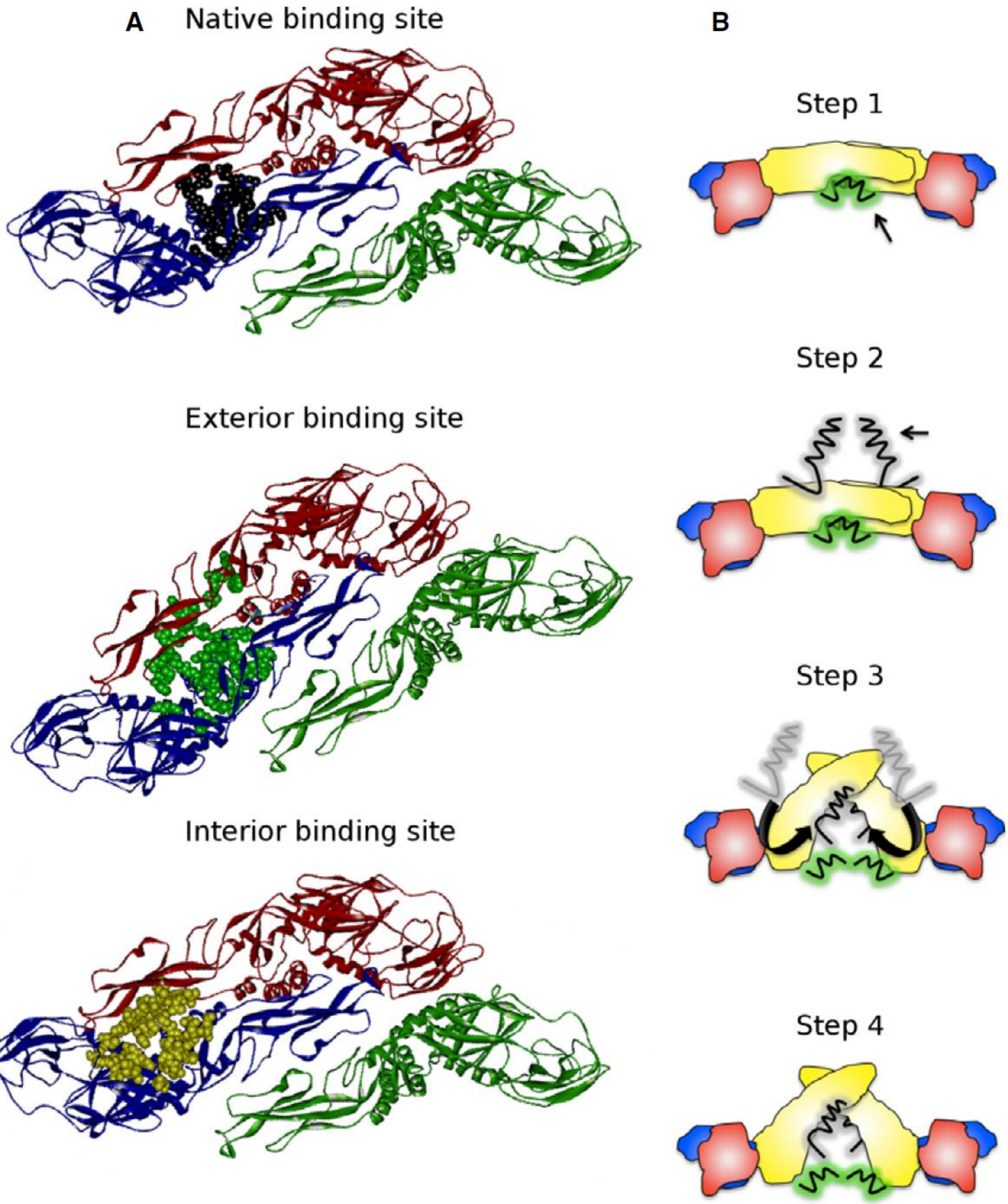

Figure 6: Molecular docking and proposed mechanism of MLH40 peptide interactions with DENV E protein.