Overview of Flavivirus Therapeutics

Pei-Yong Shi

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utmb Health

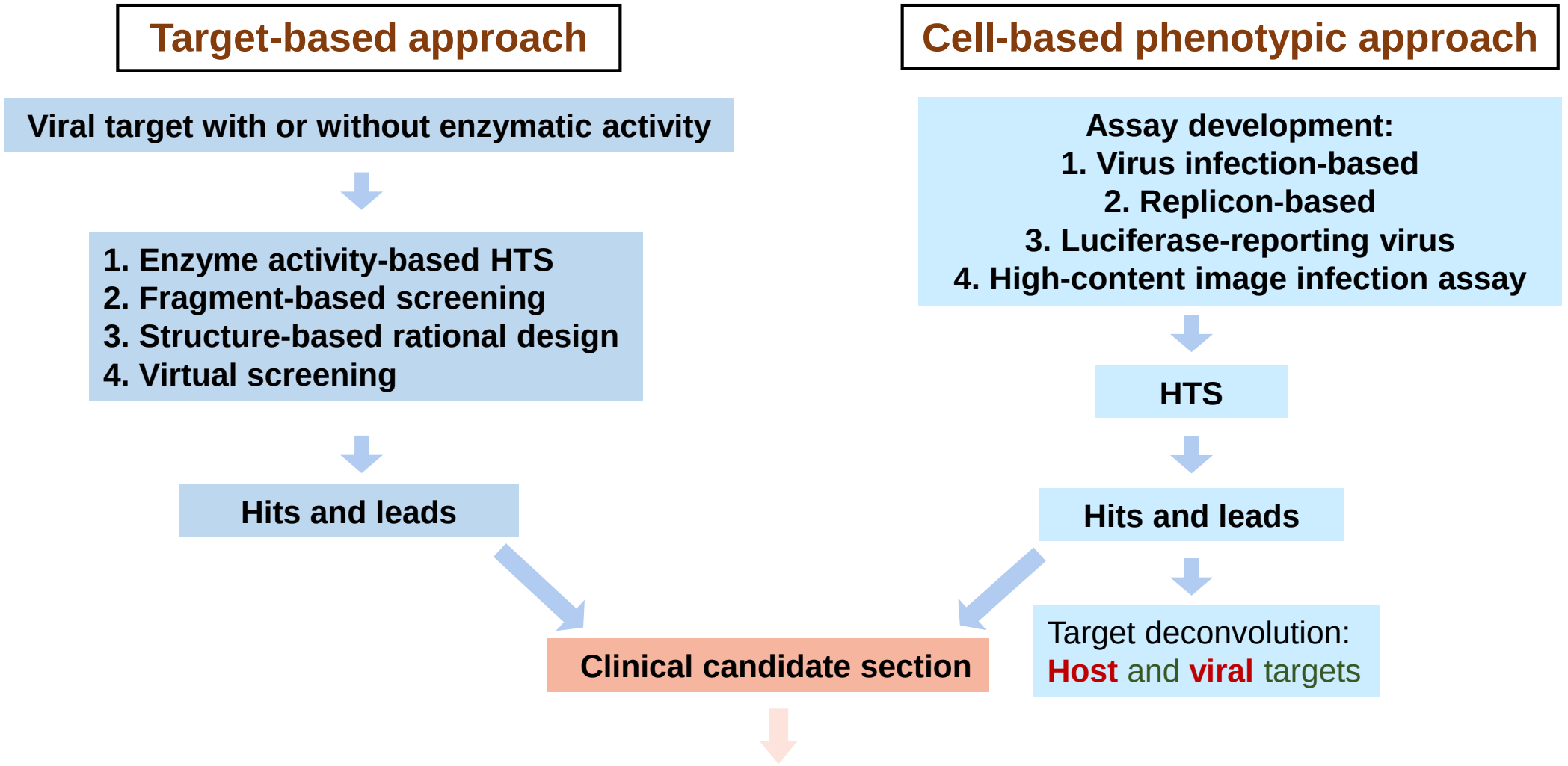
Outline

- Antiviral strategy
- Compounds tested in dengue clinical trials
- Repurposing strategy for Zika therapeutics
- Current status of dengue direct antiviral agents
- Knowledge gaps for Zika virus therapeutics

Antiviral strategy

- Targeting **viral proteins**
  - Polymerase/reverse transcriptase inhibitors
  - HIV and HCV protease inhibitors
  - HIV integrase inhibitors
  - HCV NS5A inhibitors
  - Attachment and entry inhibitors: small molecule, peptide, antibody
- Targeting **host proteins** required for viral life cycle
  - HIV CCR5 co-receptor inhibitor (Mavaviroc)
- Stimulating **immune system**
  - Interferon
  - Immune modulators: RIG-I, MDA5, Sting, and TLR modulators
- Targeting **molecular pathways** that lead to diseases
  - Molecular pathways that lead to pathogenic diseases should be clearly defined for therapeutic intervention.

Flavivirus antiviral approach



Compounds tested in dengue clinical trials: Repurposing strategy

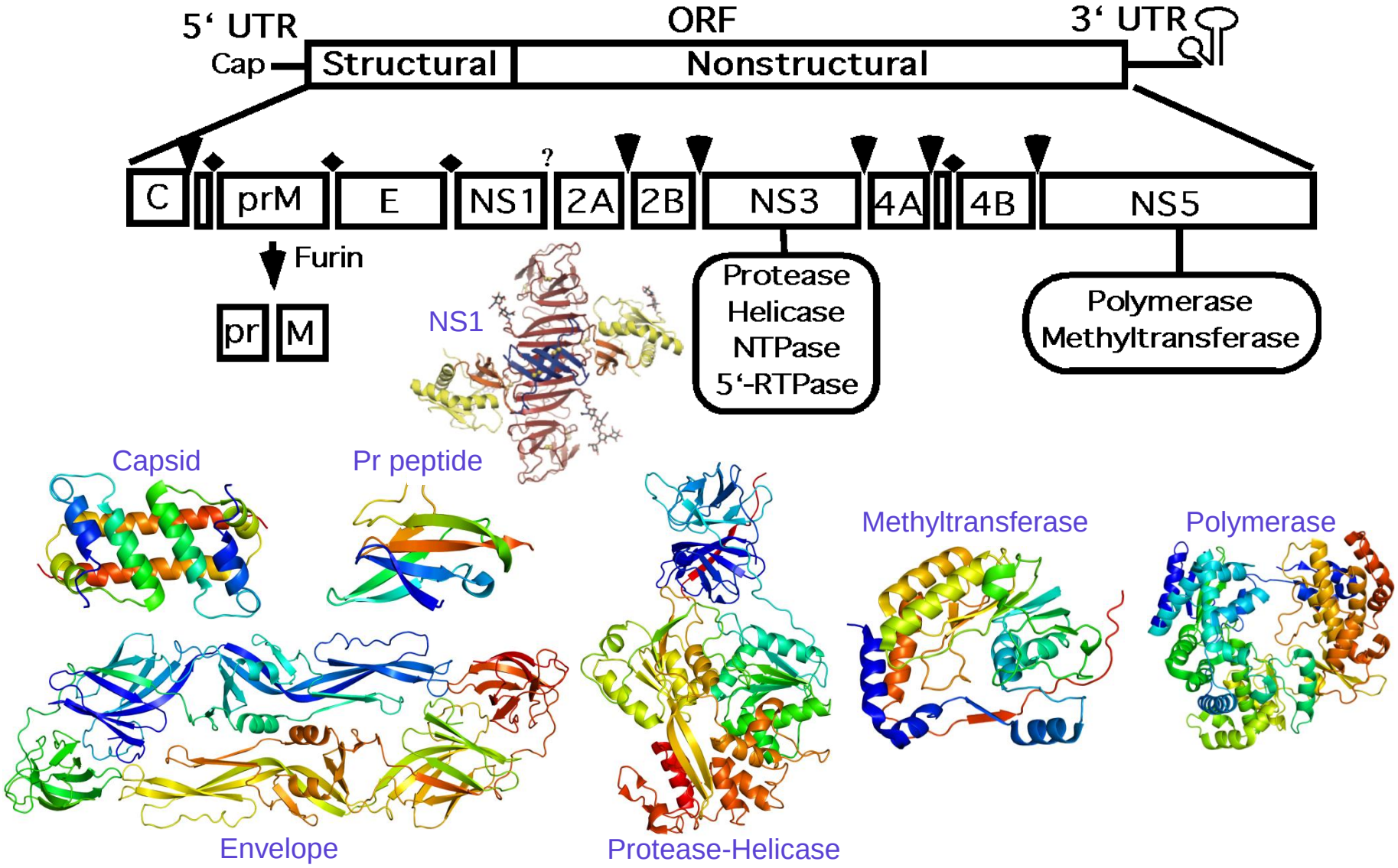
- Inhibit **viral targets**
  - Balapiravir (J Infect Dis 2013 207(9):1442): excess cytokine production triggered by dengue virus infection prevented the conversion of the balapiravir prodrug to its active form (JVI 83:1740)
- Inhibit **host targets** that are essential for viral infection cycle
  - Chloroquine: inhibitor of fusion and virion maturation, and modulator of host response to viral infection (PLoS Negl Trop Dis 2012 doi:10.1371)
  - Celgosivir and iminosugar: α-glucosidase inhibitor (Lancet Infect Dis 2014 14:706)
  - Lovastatin: cholesterol synthesis inhibitor and inflammation modulator (Clin Infect Dis 2016 62:468)
- Block the **pathological pathways** that lead to severe dengue diseases (DHF/DSS)
  - Corticosteroid: inflammation inhibitor (Clin Infect Dis 2012 55:1216)
  - Ketotifen: antihistamine mast cell stabilizer to treat vascular leakage (ongoing)

No *bona fide* inhibitor specifically designed for dengue has ever been tested in clinics.

Repurposing strategy for Zika therapy

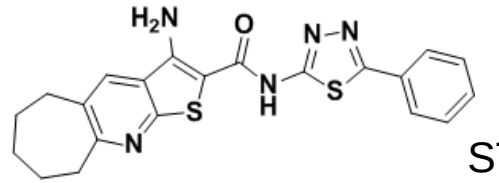
- Screening clinically approved drugs for potential Zika virus therapy should be performed.
- For any compounds active against Zika virus, does the compound exposure reach efficacious concentration (e.g., >EC_{90}) in humans ?
  - Human pharmacokinetic data are usually available for clinical compounds.
- Is the repurposed compound fast acting?
  - Fast-acting inhibitors are required to treat acute infections.
- Does pre-infection of Zika virus in patients affect compound potency?
  - Viral infection changes cell physiology that may affect compound potency, especially when the compound is a pro-drug that requires host enzyme metabolism.
- Can the compound reach viral replication sites in humans? What are the cell types that Zika virus infects?
  - Is the compound’s tissue/organ distribution known?

Flavivirus antiviral targets

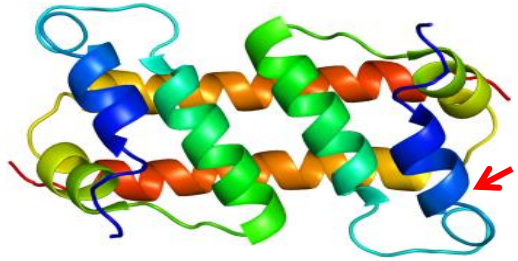


Dengue: direct antiviral agents (I)

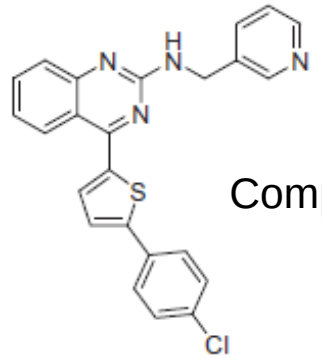
- **Capsid**: compound ST148; submicromolar EC_{50}; active in dengue AG129 mice (AAC 2013 57:15)
  - Issues: poor solubility and physical chemical property (e.g., solubility); poor formulation and bioavailability



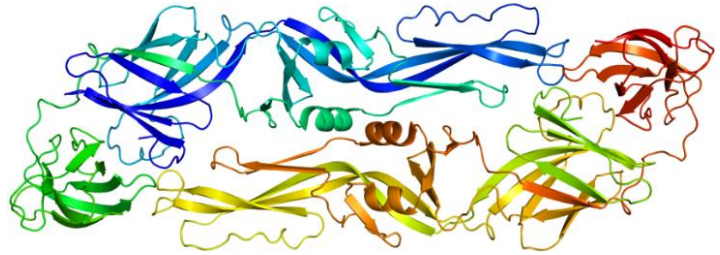
ST148


S34L

- **Envelope**
  - Therapeutic antibodies: pan-serotype activity, resistance, and cost of therapy in developing countries
  - Small molecules: target a pocket between domain-I and domain-II; submicromolar EC_{50} poor solubility (e.g., compound 6); no in vivo activity (AAC 2009 53:1823)
  - Peptide inhibitors: block envelope stem region folding-back (similar to Enfuvirtide/T20 in HIV); however, flavivirus fusion occurs in endosome, so the peptides need enter cells before exerting antiviral activity

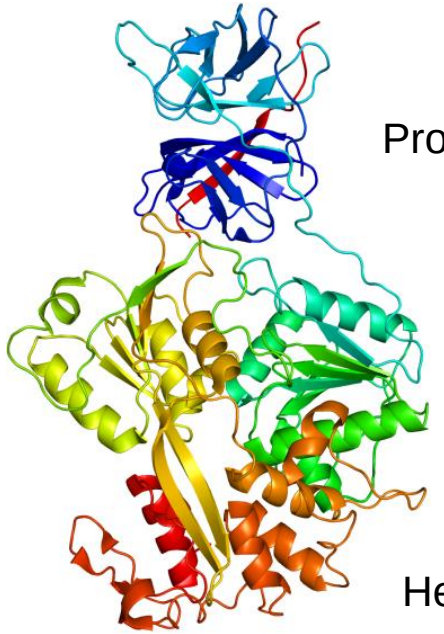


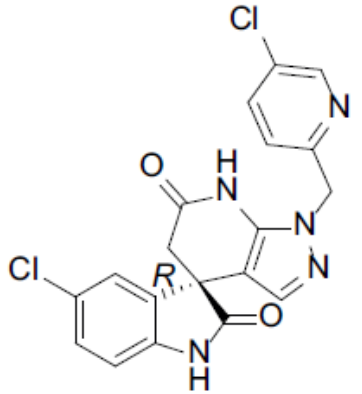
Compound 6



Dengue: direct antiviral agents (II)

- **Protease**: flat substrate-binding surface with two positively charged P1 and P2 residues, making rational design of inhibitors challenging; protease inhibitors with low activity, especially in cell culture
- **Helicase**: flat substrate binding surface, making it challenging for identifying antiviral inhibitors
- **NS4B**: the most common viral target with hits identified from cell-based phenotypic screens. Some compounds have good drug-like properties and in vivo activity (e.g., compound 14a).
  - Issues: 1. Lack pan-serotype activity for compounds with good drug-like properties (JVI 2015 89:8233); 2. Lack drug-like properties for compounds with pan-serotype activities (JVI 2011, 85:11183)



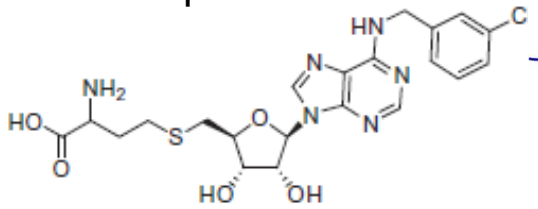


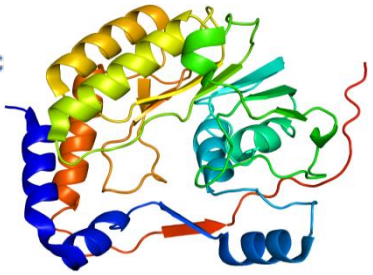
Compound-14a

Dengue: direct antiviral agents (III)

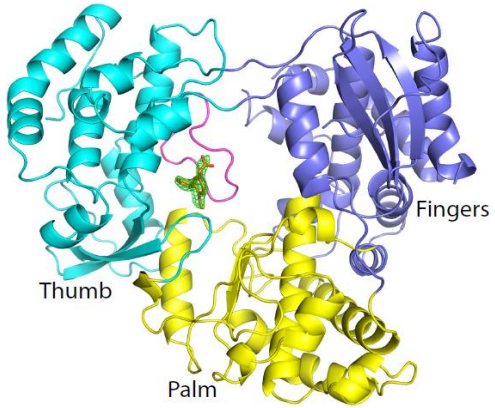
- **Methyltransferse**: selective inhibitors of flavivirus methyltransferase (e.g., compound 10) could be achieved through rational design, but these inhibitors lack cell permeability (JBC 2011 286:6233)

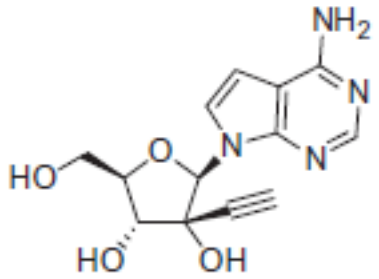
Compound 10





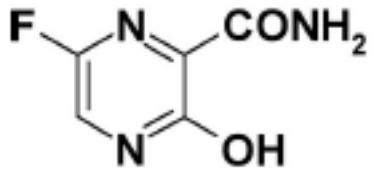
- **Polymerase**
  - **Non-nucleoside inhibitors**: compounds with nanomolar IC_{50} against dengue polymerase have been achieved; weak cellular activity due to low cell permeability (JBC 2016 in press)
  - **Nucleoside inhibitors**: NITD008 showed cell culture and mouse model activities, but failed in preclinical safety (PNAS 2009 106:26435).





NITD008

T-705 (Favipiravir)



T-705

T-705 is active against yellow fever virus in cell culture and in animal model (AAC 2009 53:202).

Knowledge gaps for Zika virus therapeutics

- Understand Zika diseases
  - Replication sites in humans (organ, tissue, and cell types) will determine (i) cell culture system that should be used for drug discovery; (ii) compound distribution in vivo during drug discovery
  - Kinetics of viral infection and disease development in patients determine the window of treatment.
- Point-of-care diagnostics to differentiate infections between Zika, dengue, and Chikungunya viruses.
- Good animal model that recapitulates Zika diseases in humans.
- How to develop antiviral compounds in pregnant patients?