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Nieuwe ontwikkelingen in de immunopathogenese van Dengue:

Belang van moleculaire diagnostiek

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Symposium "Moleculaire Diagnostiek in het AZP"

Paramaribo, 12 november 2011

Molecular Virology

University Medical Center Groningen

Modernisering van het Dengue surveillance systeem in Suriname (ZG007)

Uitvoeringsorganisatie Twinning Suriname-Nederland

Consortium van “Academisch Ziekenhuis Paramaribo”, “Bureau Openbare Gezondszorg”, “SIPOH” en Universitair Medisch Centrum Groningen

Dengue: High Impact

According to WHO estimates:

- 2.5 billion people at risk of acquiring dengue
- 50 to 100 million new cases of dengue virus infection each year
- 500,000 – 1,000,000 individuals develop DHF
- ~ 20,000 deaths

"disease of children"

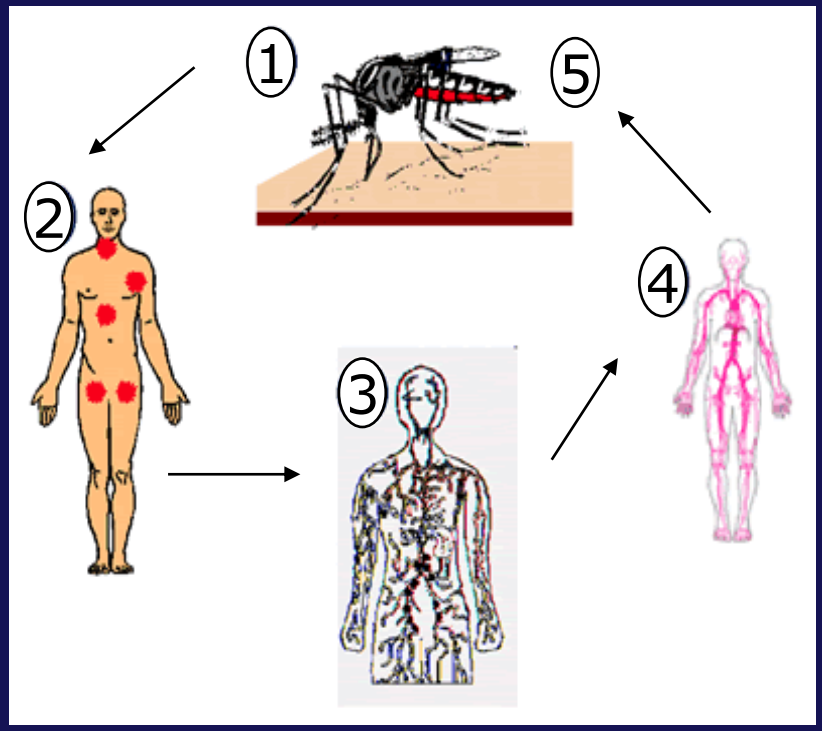
Dengue Virus

- 50-100 million infections annually
- Transmitted by *Aedes* mosquitoes
- 4 serotypes (DENV 1-4)
- All serotypes can cause 4 clinically defined manifestations

Close-up of an Aedes mosquito

Transmission cycle

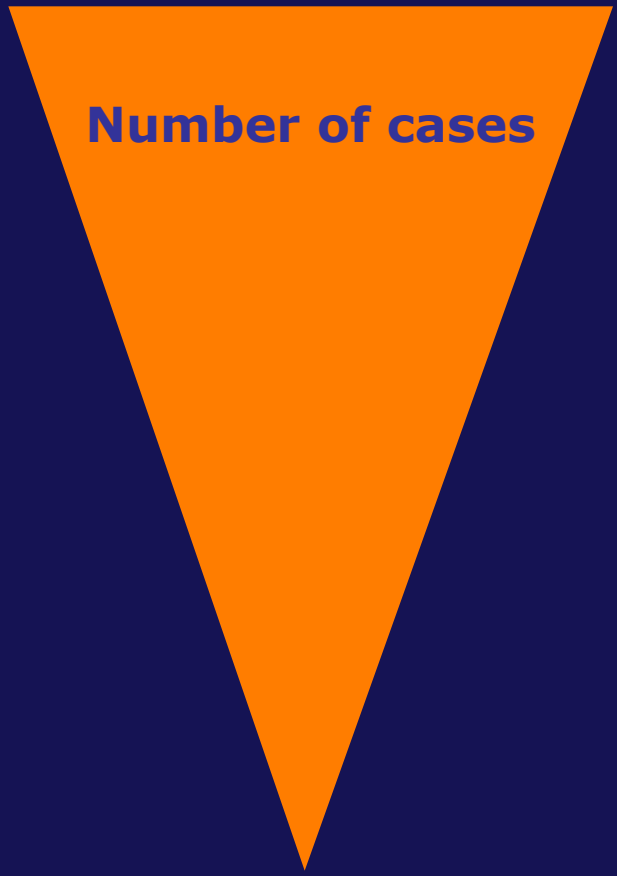
1. Transmission
2. Virus replication
3. Dengue virus infects
   - Monocytes
   - Macrophages
   - Dendritic cells
4. Virus released and circulates in blood
5. Second mosquito ingests virus with blood



Clinical syndromes

- Undifferentiated fever
- Classic Dengue Fever (DF)

  ("knokkelkoorts")
- Dengue Hemorrhagic Fever (DHF)
- Dengue Shock Syndrome (DSS)



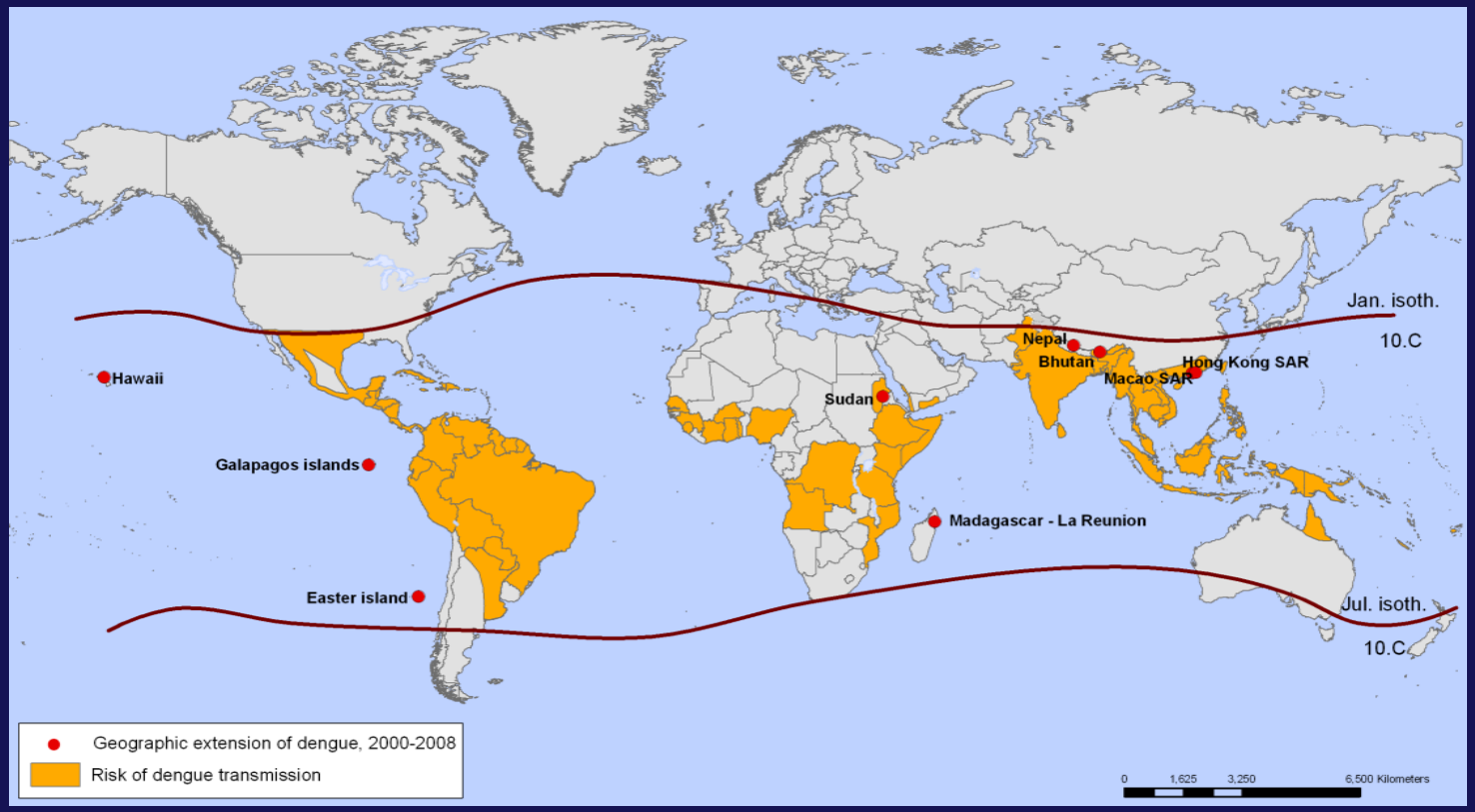
Transmission: mosquito-borne disease

Real size: 5 mm

- Transmission via mosquitoes
  - *Aedes aegypti* ("tijgermug") ► most important vector for Dengue virus
  - *Aedes albopictus*, *Aedes polynesiensis*

Dengue

Most common arthropod-borne viral infection

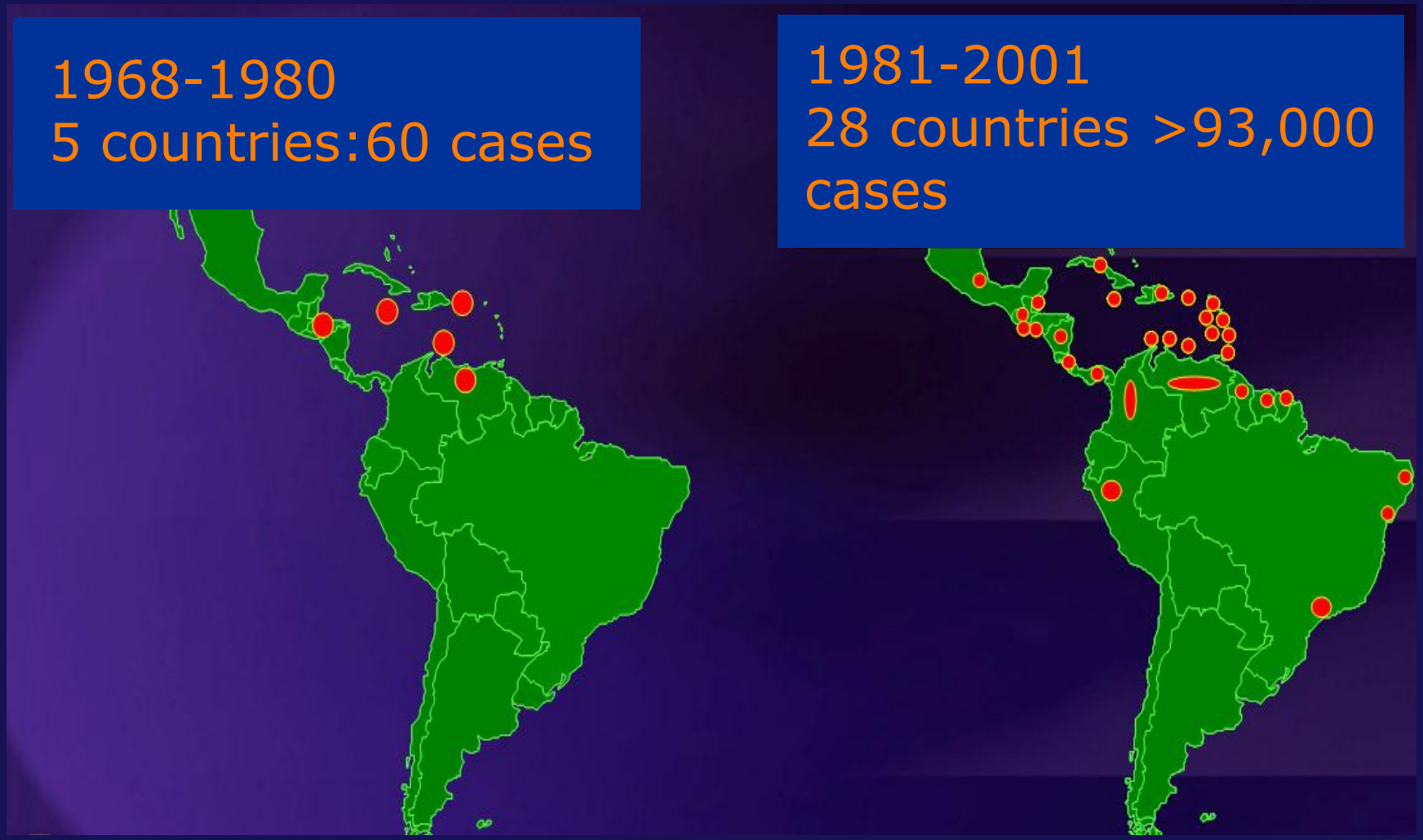


All 4 DENV serotypes circulate in the (sub) tropical regions of the world

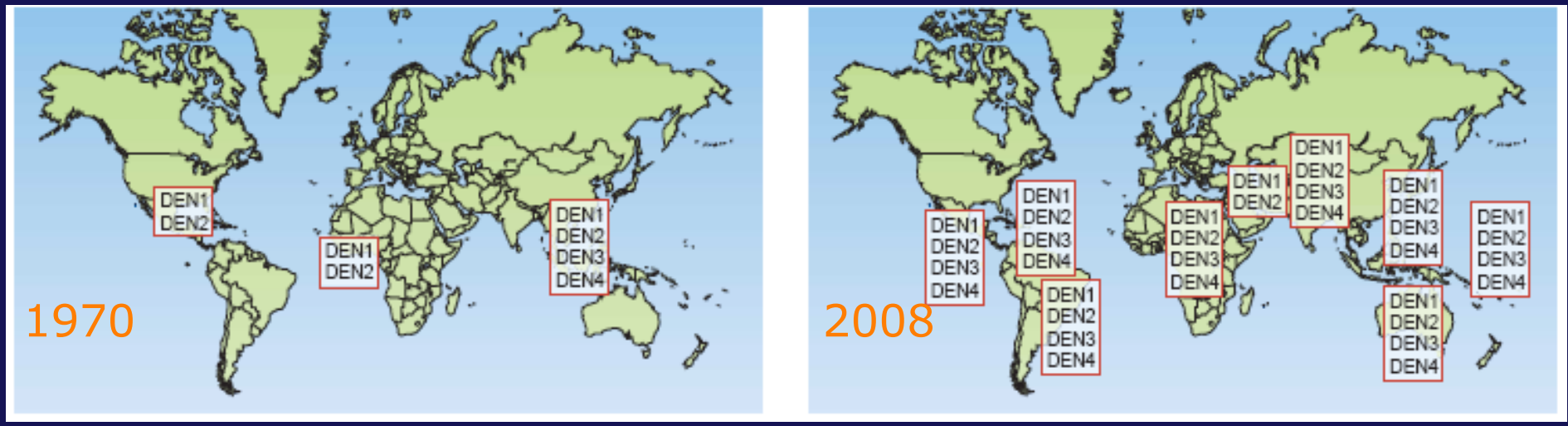
Dengue Hemorrhagic Fever

1968-1980
5 countries:60 cases

1981-2001
28 countries >93,000 cases



Recent re-emergence of dengue:
cocirculation of serotypes



Several factors are involved in emergence of dengue virus:

1.Societal/demographic; 2. Economic; 3. Ecological; 4. Virus and vector

Expectation:

More frequent and larger epidemics associated with severe disease

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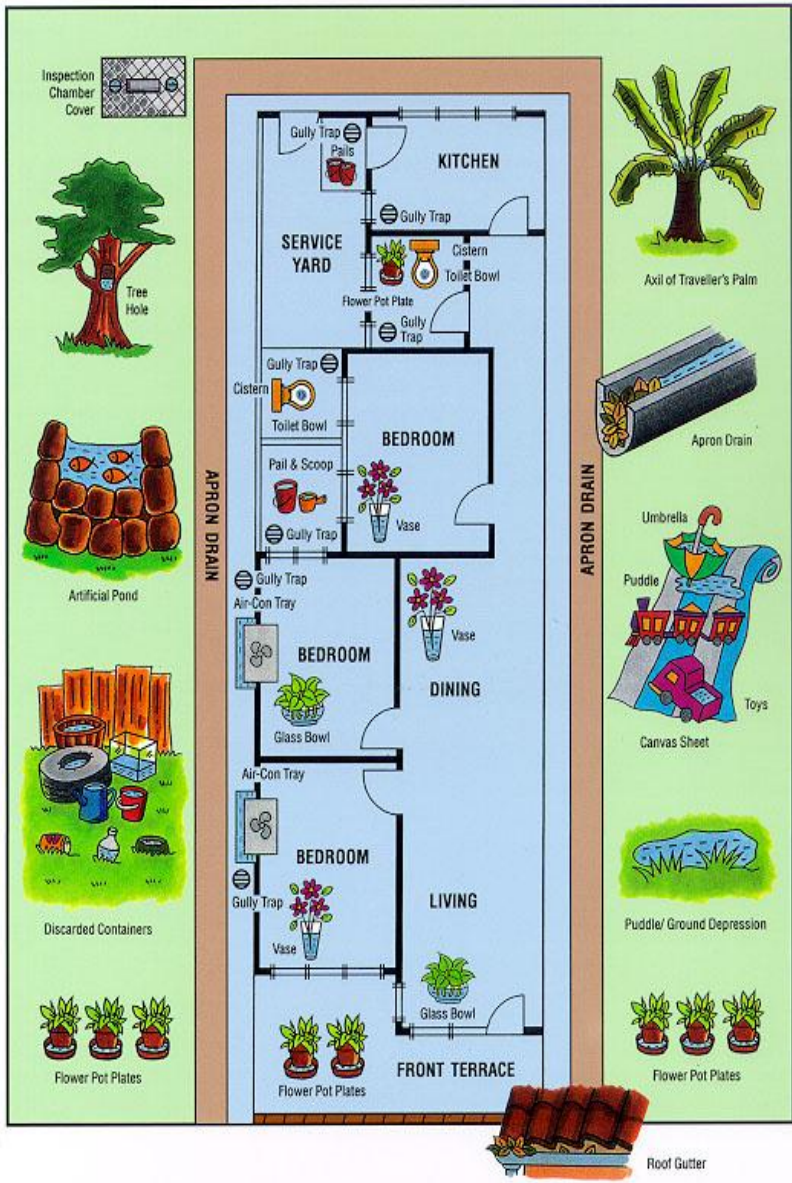
Current strategy to combat DENV : Vector Control



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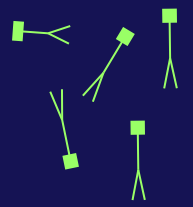
Disease and pathogenesis

1st DENV infection

DENV-1 → DF → recovery → DENV specific antibodies + memory T cells



Homotypic re-infection

DENV-1 → No disease → Pre-existing antibodies **neutralize** secondary infection

Heterotypic re-infection

DENV-2 → DF, DHF, DSS → Pre-existing antibodies may **enhance** secondary infection

ADE: Antibody-dependent enhancement

Why is the antibody response to a previous infection responsible for severe disease?

- Infants born to dengue-immune mothers are susceptible to severe disease during a primary infection
- This susceptibility disappears at approximately 1 year of age
- This indicates that, in these infants, maternal antibodies are involved in the development of severe disease during a primary infection

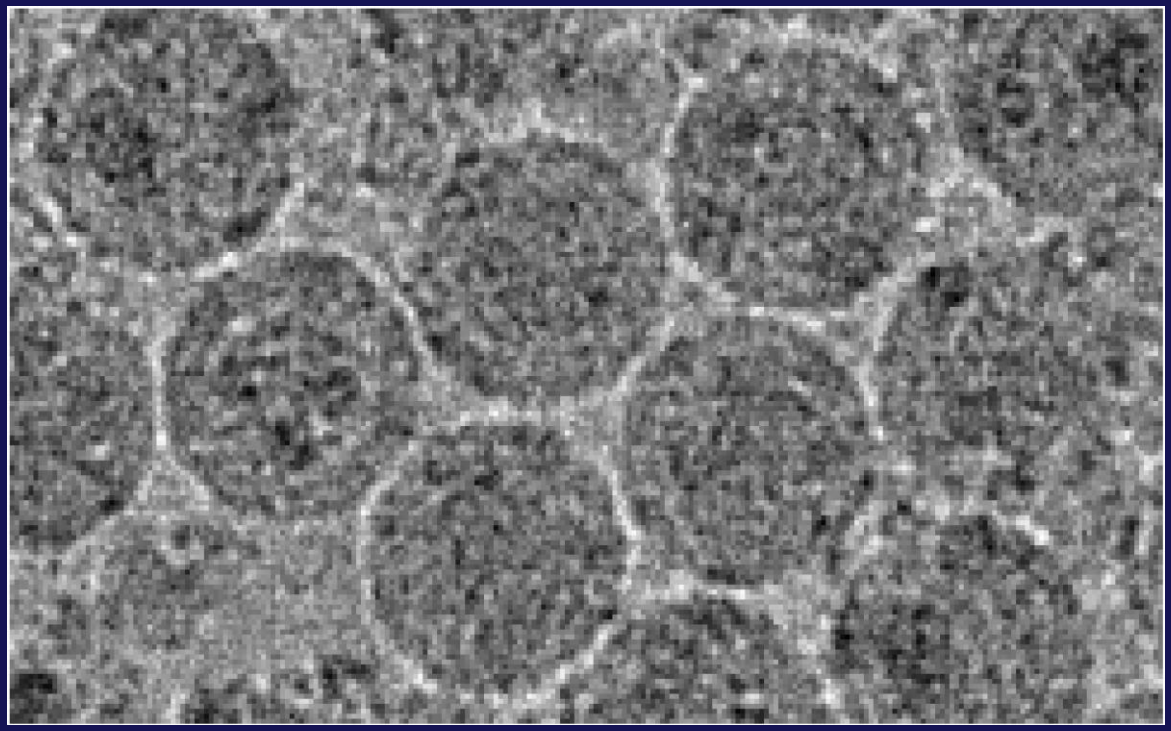
Critical aspects of the immunopathogenesis of DHF

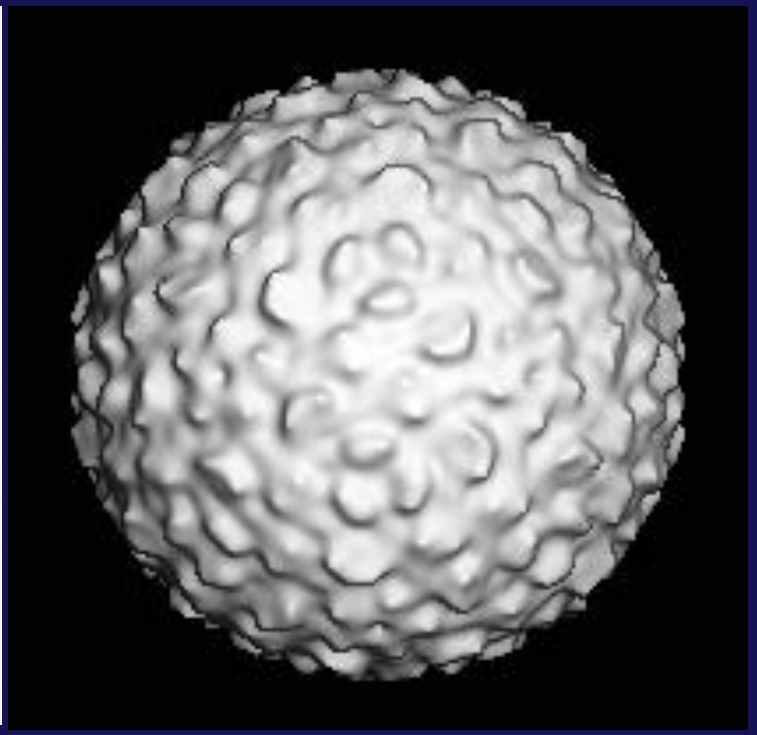
- Pre-existing heterotypic antibodies
- Enhanced uptake of virus by Fc-receptor-bearing cells (monocytes, macrophages and DCs)
- Large infected cell mass and high viral load early in infection
- Strong immune response and release of cytokines
- Endothelial cell damage and hemorrhagic manifestations (primarily plasma leakage and hemoconcentration)

Risk Factors for DHF

- Pre-existing anti-dengue antibody
  - previous infection with another DENV serotype (1-4)
  - maternal antibodies in infants
- Virulence of the virus strain
- Host
  - genetic background (ethnicity)
  - age

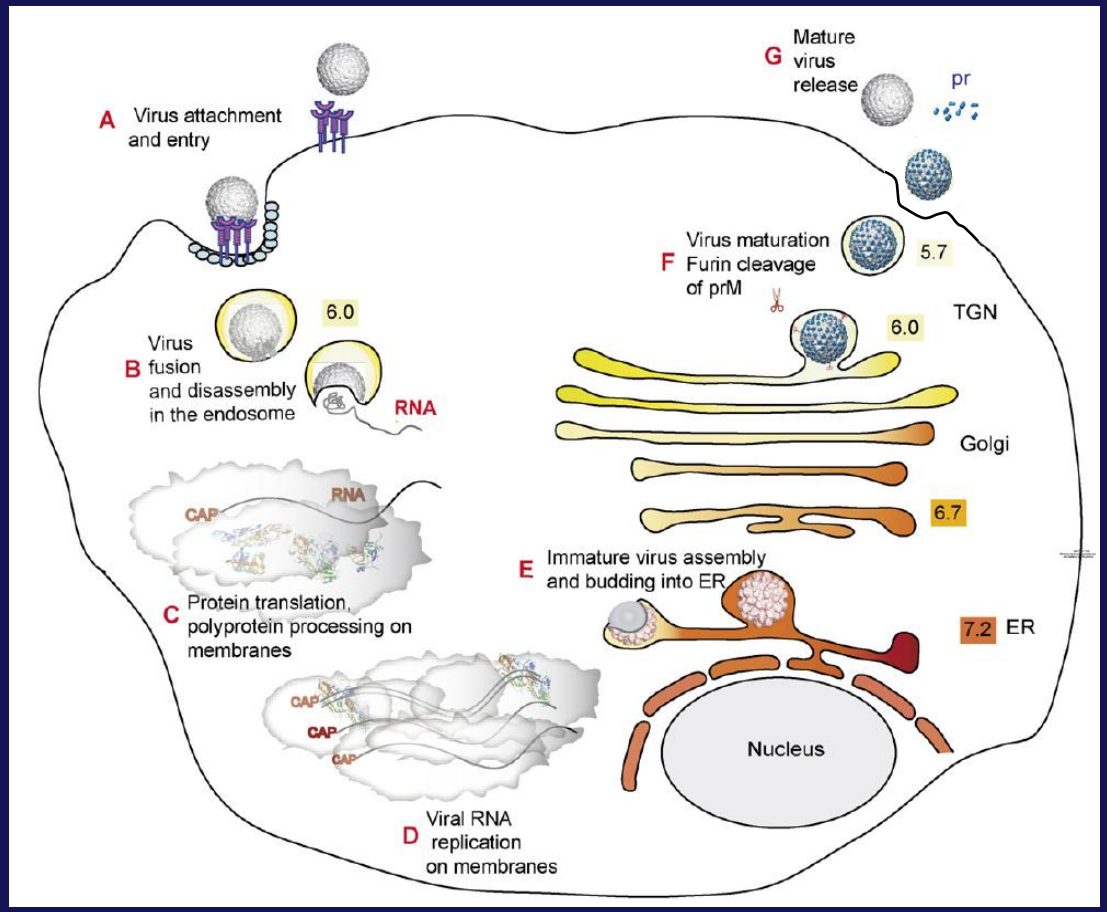
Structure of dengue virus:
cryo-EM image reconstruction

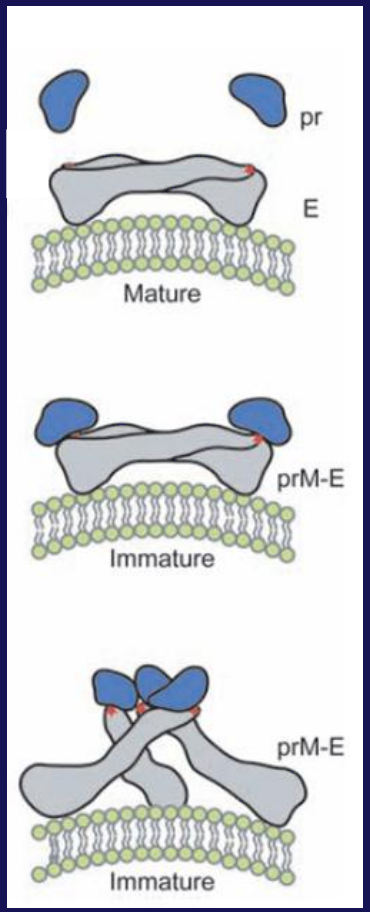


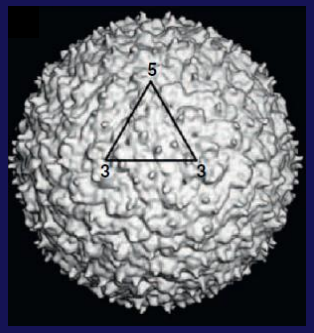


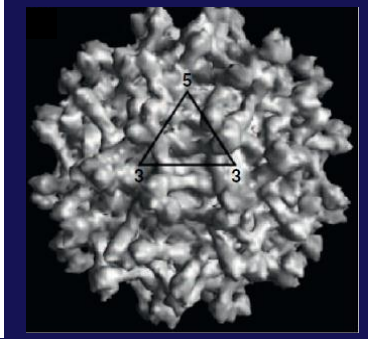
(Kuhn *et al.*, Cell, 2002, 108, 717-725)

Cellular life cycle of dengue virus

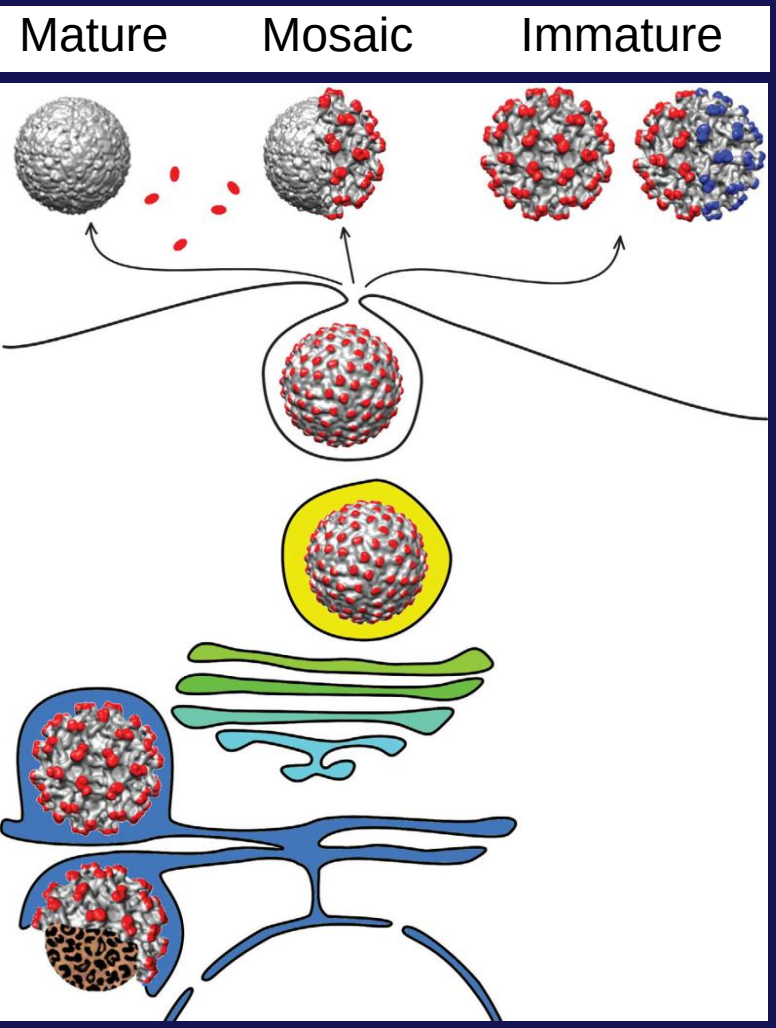




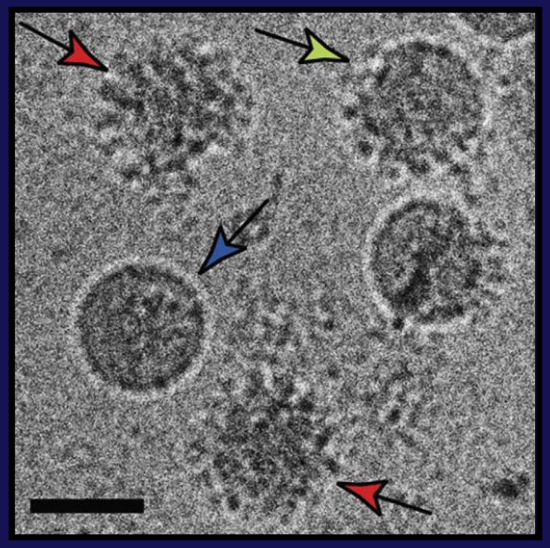




Assembly of Flavivirus particles



Flavivirus-infected cells release a high proportion of particles containing prM



Mature

Partially mature

Immature

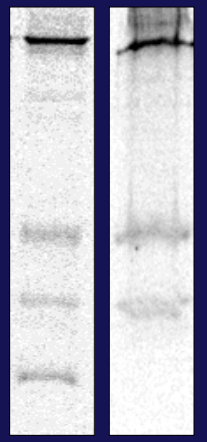
Plevka *et al.*, EMBO Reports (2011), Cherrier *et al.*, EMBO Journal (2009)

Immature DENV virus

- Furin-deficient LoVo cells secrete fully immature DENV

  prM content

  - C6/36-derived DENV 30%
  - LoVo-derived DENV 100%



- Immature DENV is essentially noninfectious:

Particle-to-pfu ratio

Mature	Immature
100	> 1 x 10^6

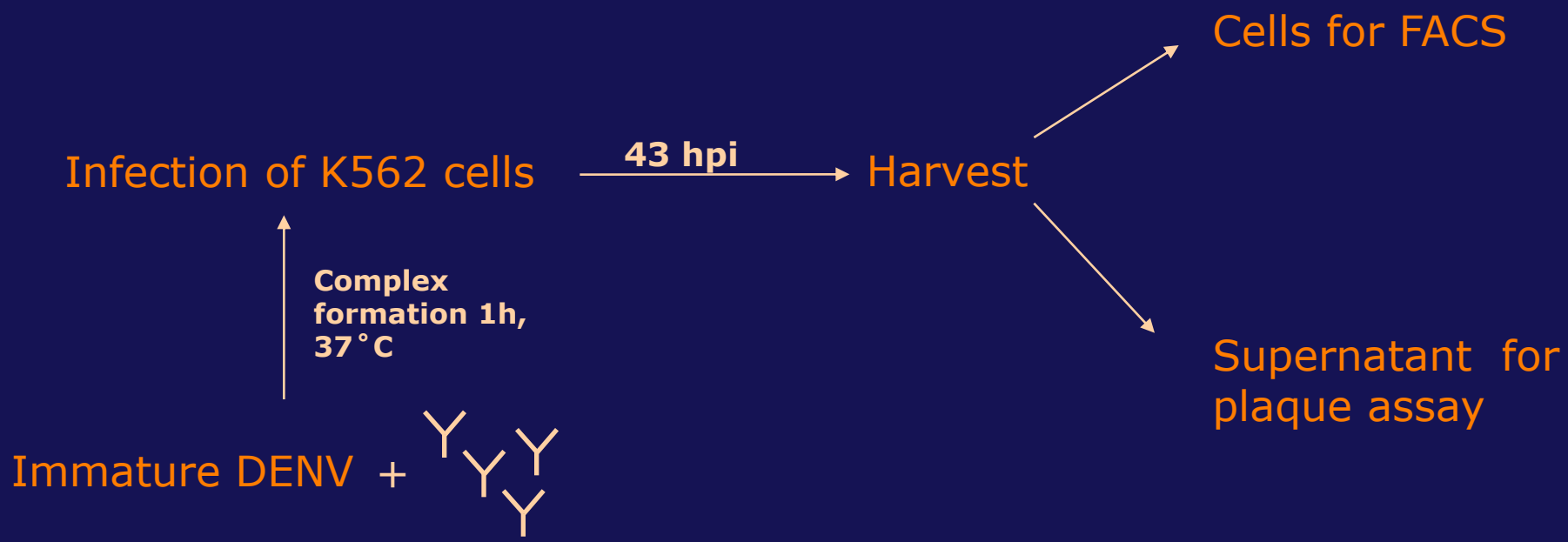
Zybert *et al*, J.Gen.Virol., 2008

Immature Dengue Virus:

A Veiled Pathogen?

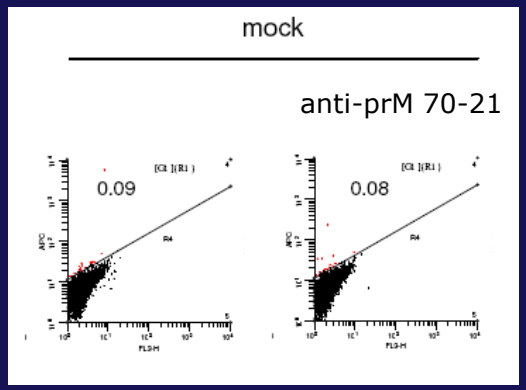
- Considerable levels of antibodies against prM are found in Dengue-positive patients' sera [Se-Thoe, *et al.,* 1999; Cardosa, *et al.,* 2002; Dejnirattisai, *et al.,* 2010; de Alwis *et al.,* 2011]
- The levels of prM antibodies are significantly higher in patients experiencing a secondary infection [Lai, *et al.,* 2008] and are significantly elevated in patients suffering from DHF/DSS [Rai, *et al.,* 2008]

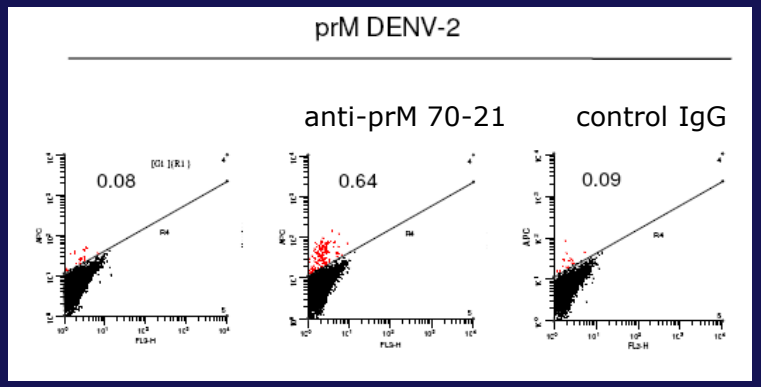
Infectivity of immature DENV in FcR-bearing cells in presence of anti-prM antibodies



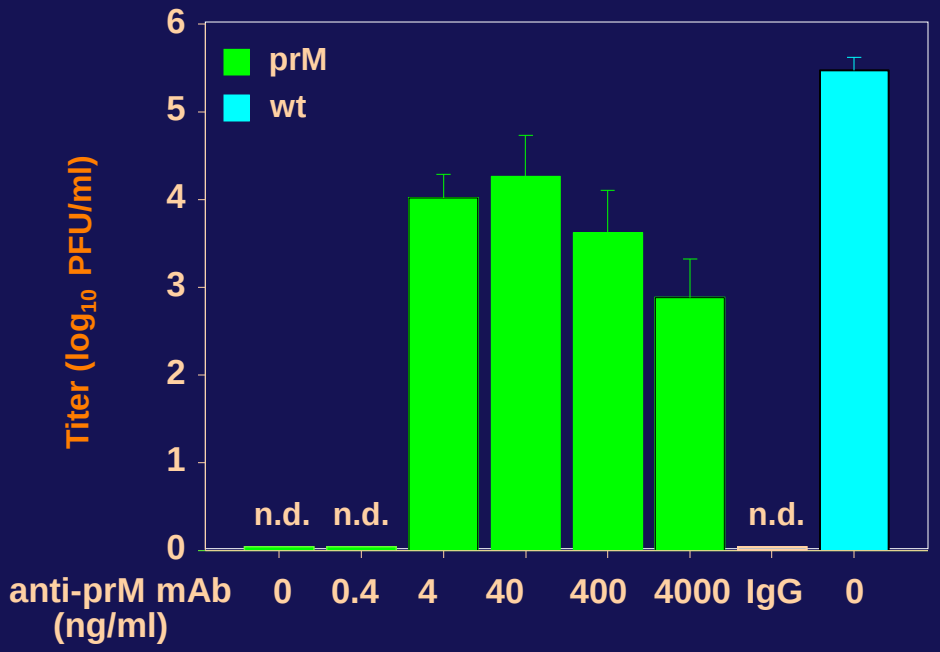
Immature virions turn highly infectious in presence of anti-prM antibody

Cells



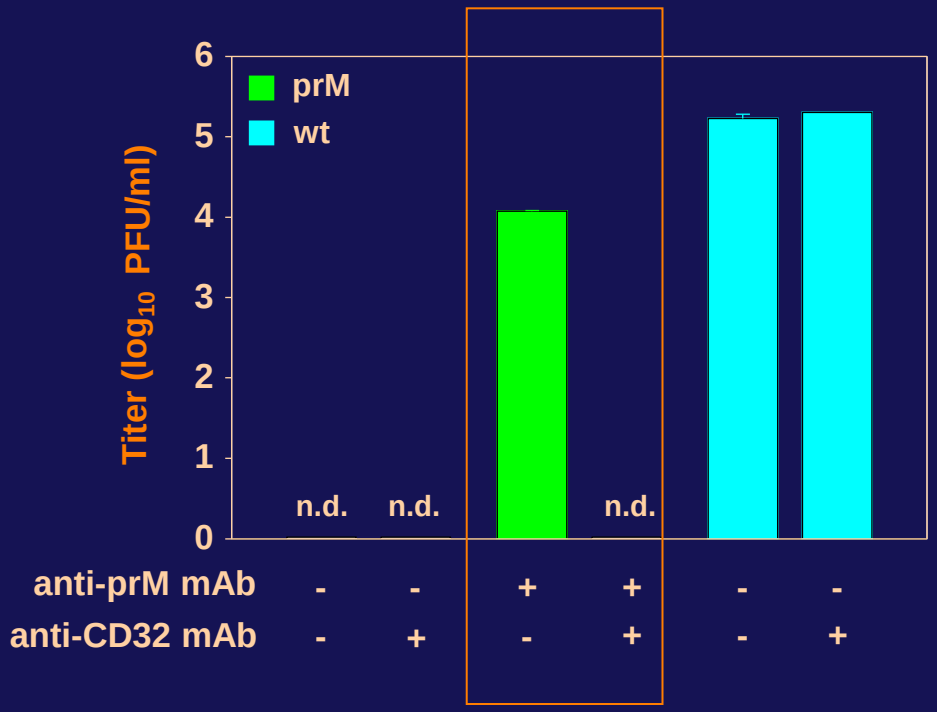


Supernatant

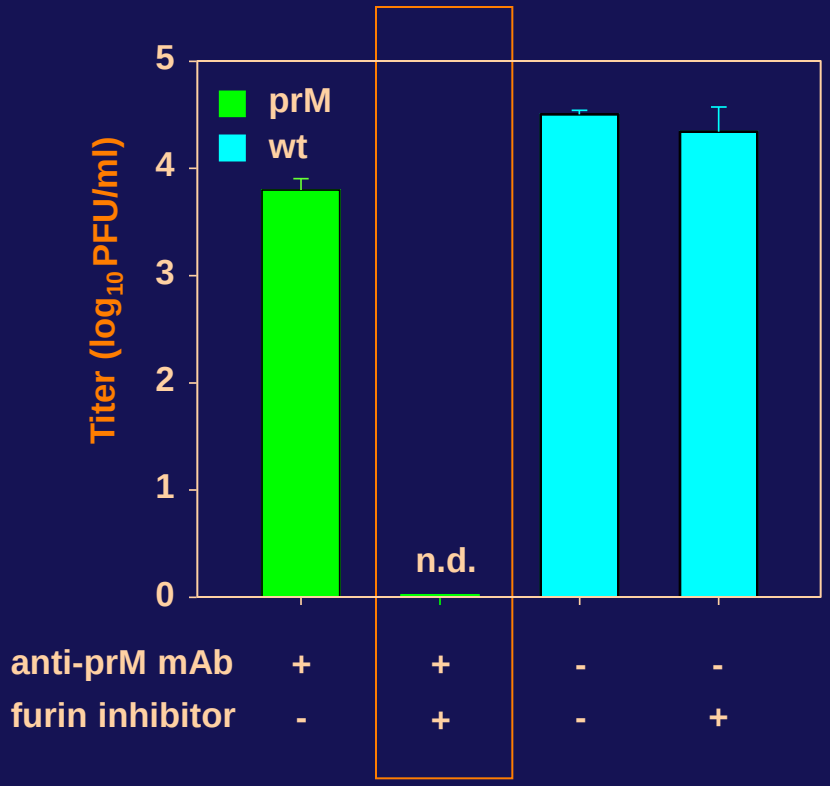


Rodenhuis-Zybert *et al.*, PLoS Pathogens, 2010

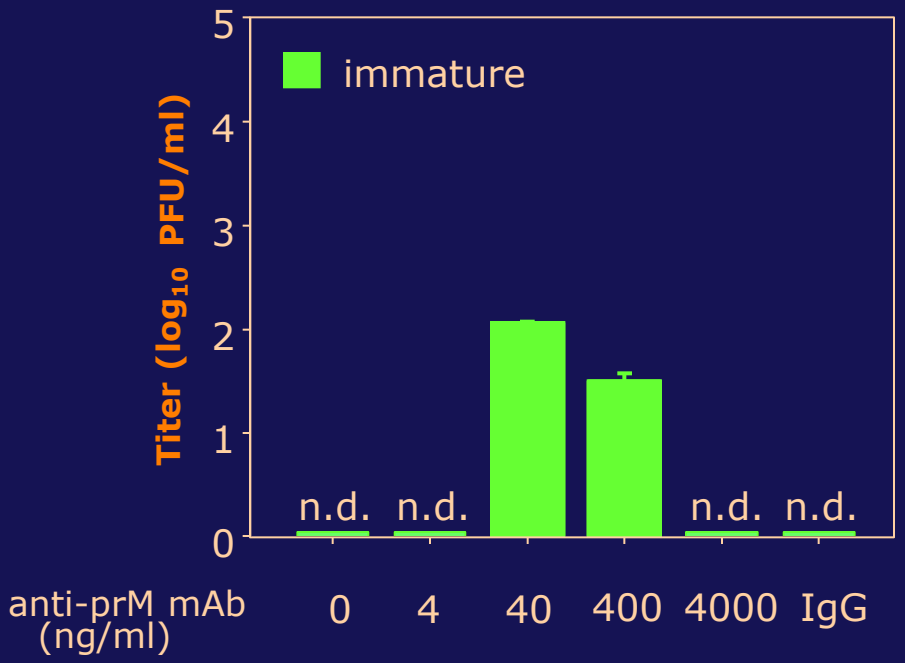
Blocking Fc-receptors impedes enhancement of infection

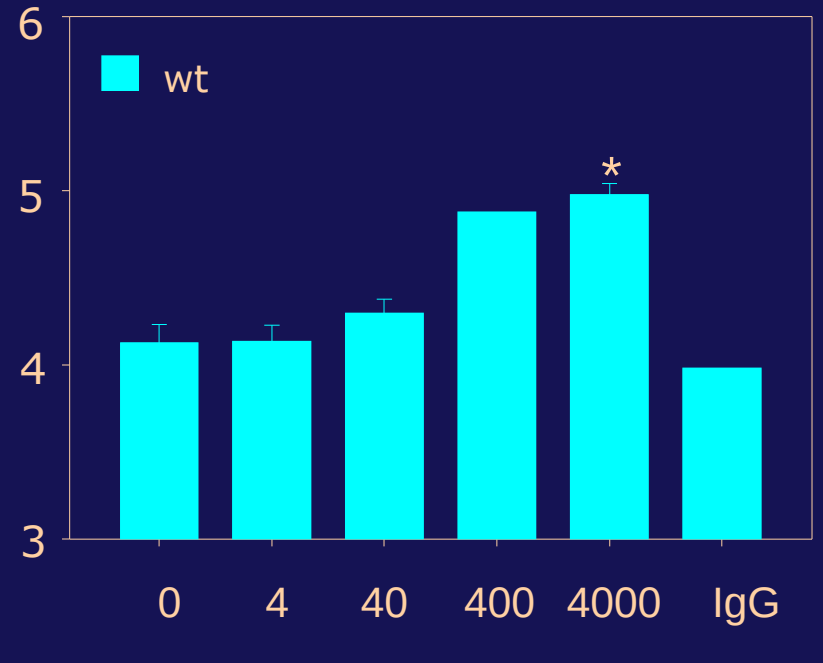


Furin activity in target cell is crucial to render immature virions infectious

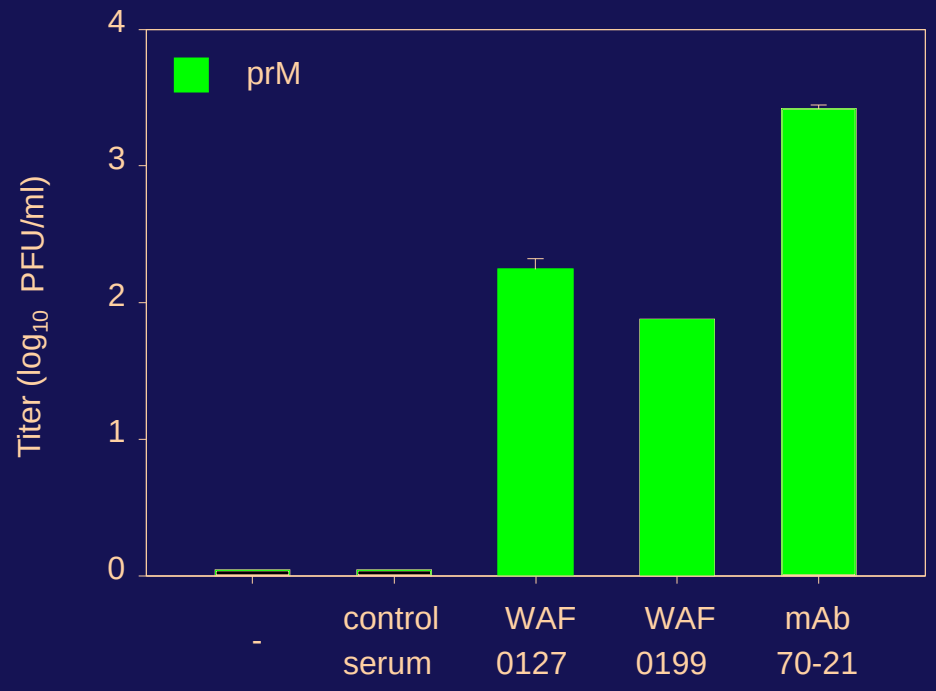


Enhancement of infection in human PBMCs of both immature and wt virus





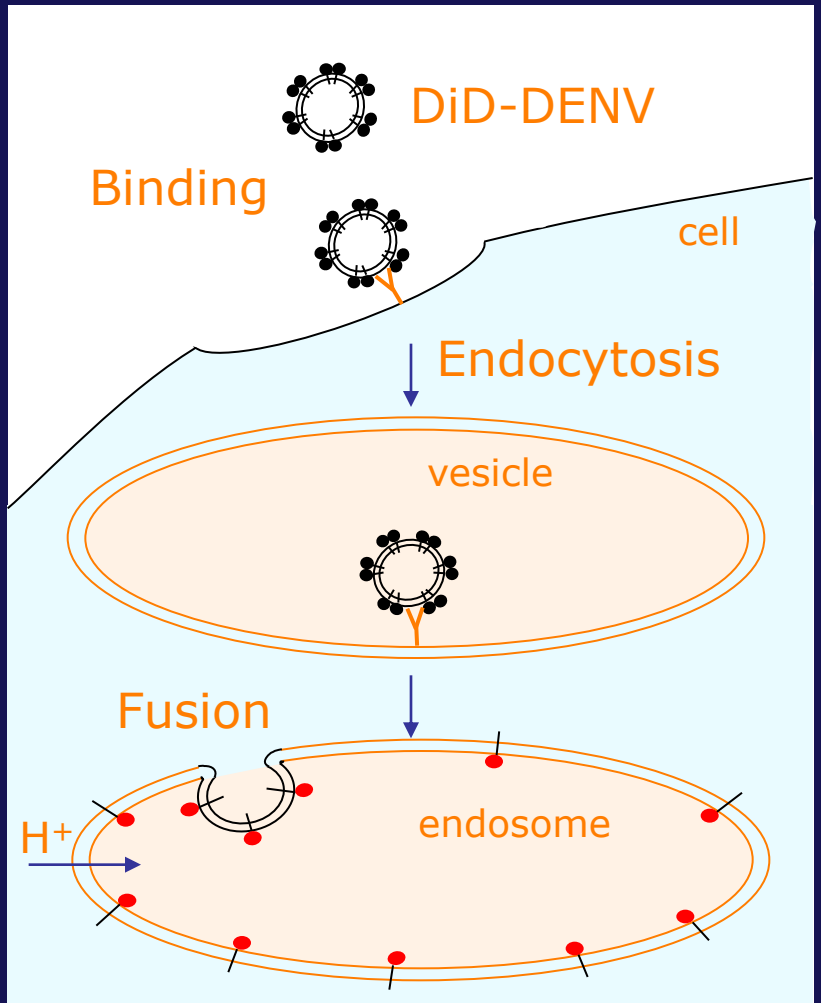
Immature DENV becomes infectious in the presence of DENV-immune sera



Sera obtained from Dr. Guillermo Comach, LARDIDEV, BIOMED-UC, Maracay, Venezuela

Visualization of dengue virus entry in living cells: single-particle tracking

- DENV is labeled with the lipophilic fluorescent probe DiD
- DiD surface density
  - High : self-quenching
  - Low : bright fluorescence



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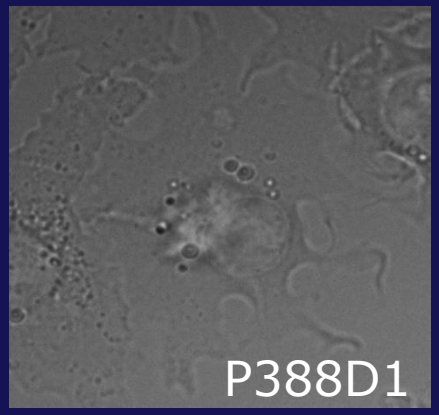
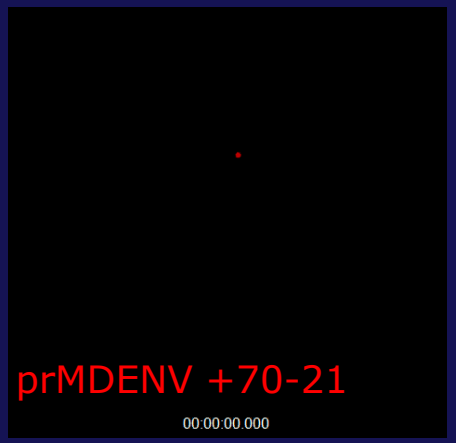
Analysis of the cell entry dynamics of prMDENV opsonized with prM antibodies

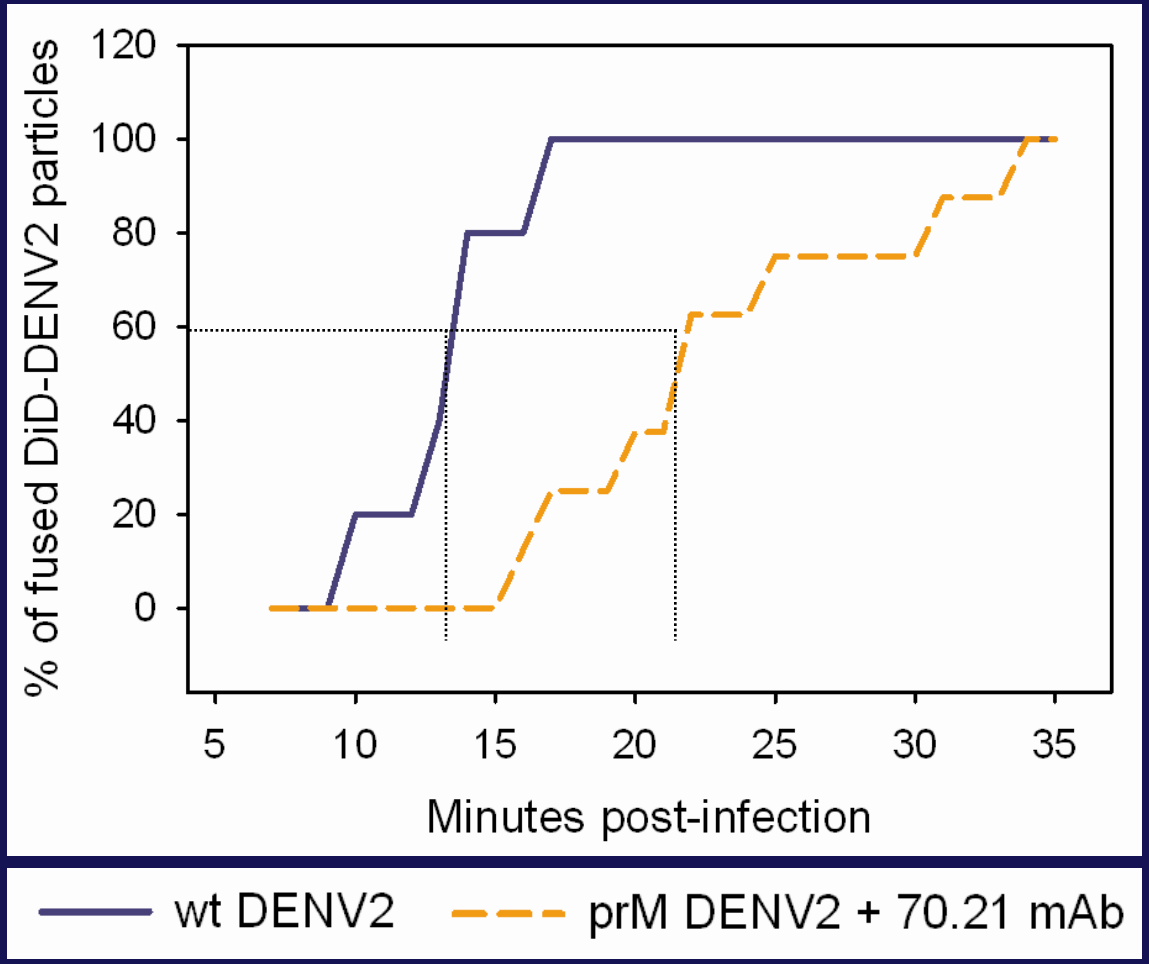




- Single-particle tracking
  - murine P388D1 macrophage cells
- Treatment of cells with chemical inhibitors indicates that prMDENV-immune complexes infect cells through phagocytosis

Ayala-Nunez *et al*, unpublished

Immature DENV fuses at a later timepoint than standard DENV



Ayala-Nunez *et al*, unpublished

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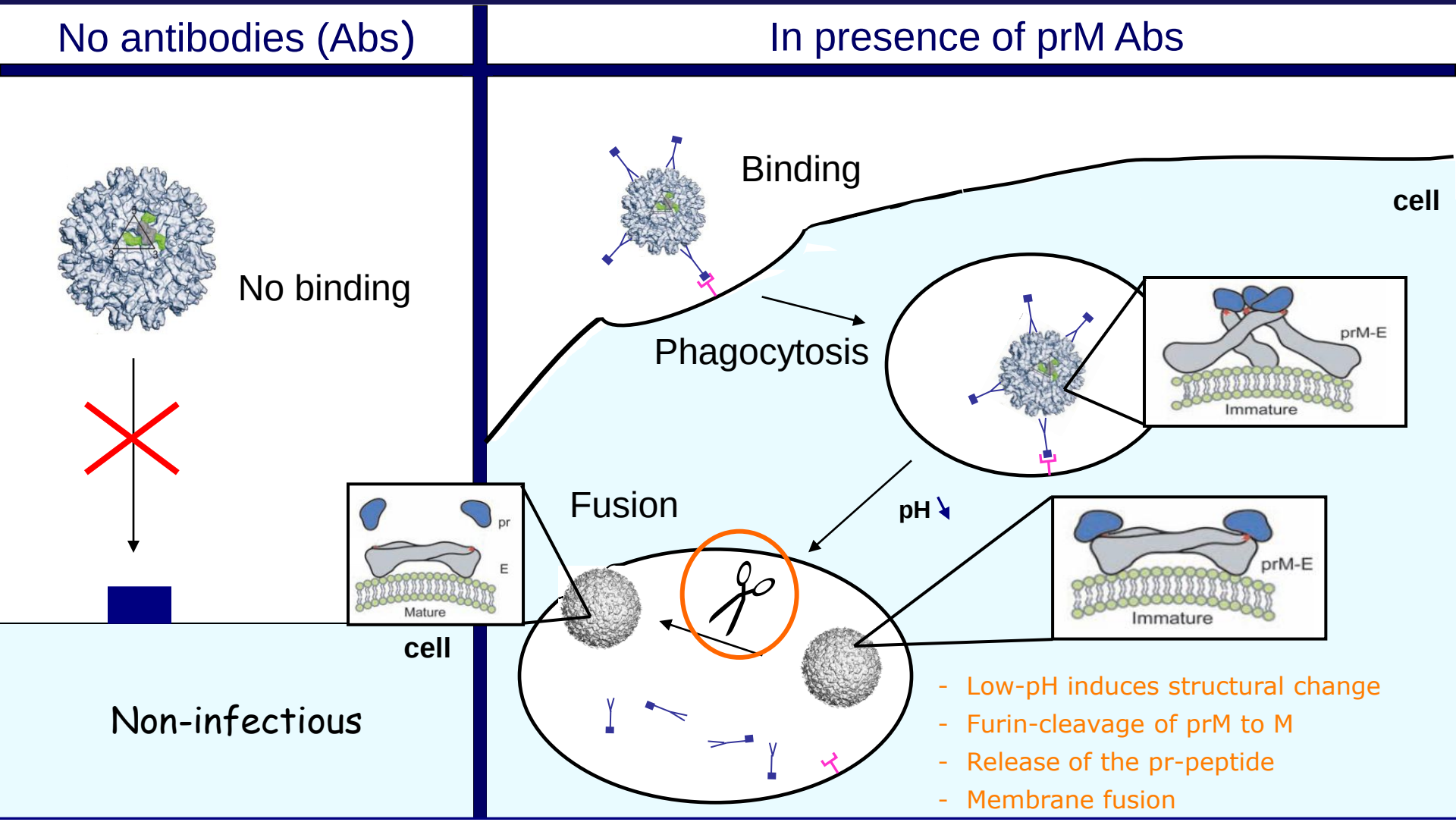
Model of immature flavivirus entry to cells



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Disease and pathogenesis

1st DENV infection

DENV-1 → DF → recovery → DENV specific E, prM, NS1 antibodies + memory T cells

Homotypic re-infection

DENV-1 → No disease → Neutralization: Predominant role for serotype-specific E antibodies

Heterotypic re-infection

DENV-2 → DF, DHF, DSS → Enhancement: Predominant role for cross-reactive prM and/or E antibodies

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Perspectieven en Belang van Moleculaire Diagnostiek

- Moderne, snelle, op PCR gebaseerde, moleculaire diagnostiek is essentieel voor een adequate behandeling van patienten en zal uiteindelijke levens redden
- Een state-of-the art surveillance-systeem, gebaseerd op GIS, is cruciaal voor early warning en controle van Dengue-uitbraken
- Kennis op het gebied van de infectiemechanismen en de pathogenese van Dengue, ihb kennis van het mechanisme van ADE, is cruciaal voor de ontwikkeling van effectieve en veilige vaccins en antiviralen middelen en identificatie van predictors of severe disease. Moleculaire diagnostiek speelt hierin een essentiële rol
  - Infectiehistorie van patienten
  - Immuunrespons op infectie
  - Moleculaire karakterisering van de virussen die DHF/DSS veroorzaken

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Acknowledgements

University Medical Center Groningen, Groningen, The Netherlands

Izabela Rodenhuis-Zybert
Hilde van der Schaar
Bastiaan Moesker
George Sips
Heidi van der Ende-Metselaar
Adriana Tami
Jolanda Smit

Purdue University, Lafayette, USA

Richard Kuhn
Michael Rossmann

Harvard University, Cambridge, USA

Michael Rust
Xiaowei Zhuang

Washington University, St Louis, USA

Daved Fremont
Michael Diamond

PDVI Pediatric DENGUE VACCINE INITIATIVE

NWO Nederlandse Organisatie voor Wetenschappelijk Onderzoek

UTSN Project Partners

- **Academisch Ziekenhuis Paramaribo**
  - Amadu Juliana (Co-ordinator SR)
  - John Codrington
  - Jimmy Roosblad
- **Bureau Openbare Gezondheidszorg**
  - Willem Bakker
  - Marjolein Jubithana
  - Merril Wongsokaryo
  - Frans Amatali
- **SIPOH**
  - Jan van Charante
- **Universitair Medisch Centrum Groningen**
  - Adriana Tami (Co-ordinator NL)

Adviseurs en directie UTSN

- **Paramaribo**
  - Prof. Marten Schalkwijk
  - Mevr. Usha Schalkwijk-Doerga
- **Utrecht**
  - Richard Born en medewerkers

Niet te vergeten….
de administrative medewerkers

- **AZ Paramaribo**
  - Claudia Redan
  - Madelon Vlieri
- **UMCG Groningen**
  - Jolanda Oldengarm

…en de organisatoren van dit symposium

- **UTSN SOA & UTSN Dengue**
  - Mario van Berlo
  - Sandra Hermelijn
  - Amadu Juliana

- **SPAOGS Paramaribo**
  - Y. Chou-Lie
  - L. Akrum-Jong A Kiem
  - B. Ting A Kee

- **Orga-Nice**
  - Marisol van der Kuyp