Theoretical and practical aspects of flaviviral antigenic structure in diagnostic assays- Lessons from the past and promise for the future

Diagnostic Assays for Arboviruses

Serum, CSF

Serological Assays for Antibodies

- IgM ELISA
- IgG ELISA
- PRNT
- CF
- HI
- IFA
- Dipsticks

Mosquito pools, Tissues

Virus Detection Assays

- Virus isolation (cell culture, mice)
- IFA
- TaqMan RT-PCR
- Ag-capture ELISA
- RT-PCR / sequencing
- Dipsticks
- NASBA

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Approaches to Enhanced Testing

- Increase specificity
- Increase sensitivity
- Reduce sample size
- Increase speed of testing
- Multiplexing

Species in the Genus Flavivirus (family *Flaviviridae*)

Tick-borne viruses

- Mammalian tick-borne virus group (TBE, POW)
- Seabird tick-borne virus group

Mosquito-borne viruses

- Aroa virus group
- Dengue virus group
- Japanese encephalitis virus group
- Kokobera virus group
- Ntaya virus group
- Spondweni virus group
- Yellow fever virus group

Viruses with no known arthropod vector

- Entebbe bat virus group
- Modoc virus group
- Rio Bravo virus group

Tentative Species in Genus

- Tanama bat virus
- Cell fusing agent

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Members of the Japanese encephalitis virus serocomplex

Cacipacore virus
Koutango virus
Japanese encephalitis virus
Murray Valley encephalitis virus
Alfuy virus
St. Louis encephalitis virus
Usutu virus
West Nile encephalitis virus
Kunjin virus
Yaounde virus

Types of Antibody Reactivities (*e.g.*, WNV)

- **Subtype** – reacts with some but not all WNVs
- **Type** – reacts with all WNVs
- **Subcomplex** – reacts with 2 or more members of the JEV complex
- **Complex** – reacts with all members of the JEV complex
- **Subgroup** – reacts with 2 or more complexes
- **Group** – reacts with all flaviviruses

ELISA Cross-reactivities of IgM

Serum	SLE	JE	WN	DEN2	YF	POW
1	4.96	7.75	16.74	2.45	1.82	1.56
2	4.8	13.77	16.68	4.13	2.14	1.75
3	5.45	9.67	16.08	4.09	1.61	1.44
4	4.76	10.07	17.19	3.32	1.62	1.3
Positive Control	6.5	8.2	6.34	7.45	3.96	4.5

* 1:400 screening dilution of WNV patient serum

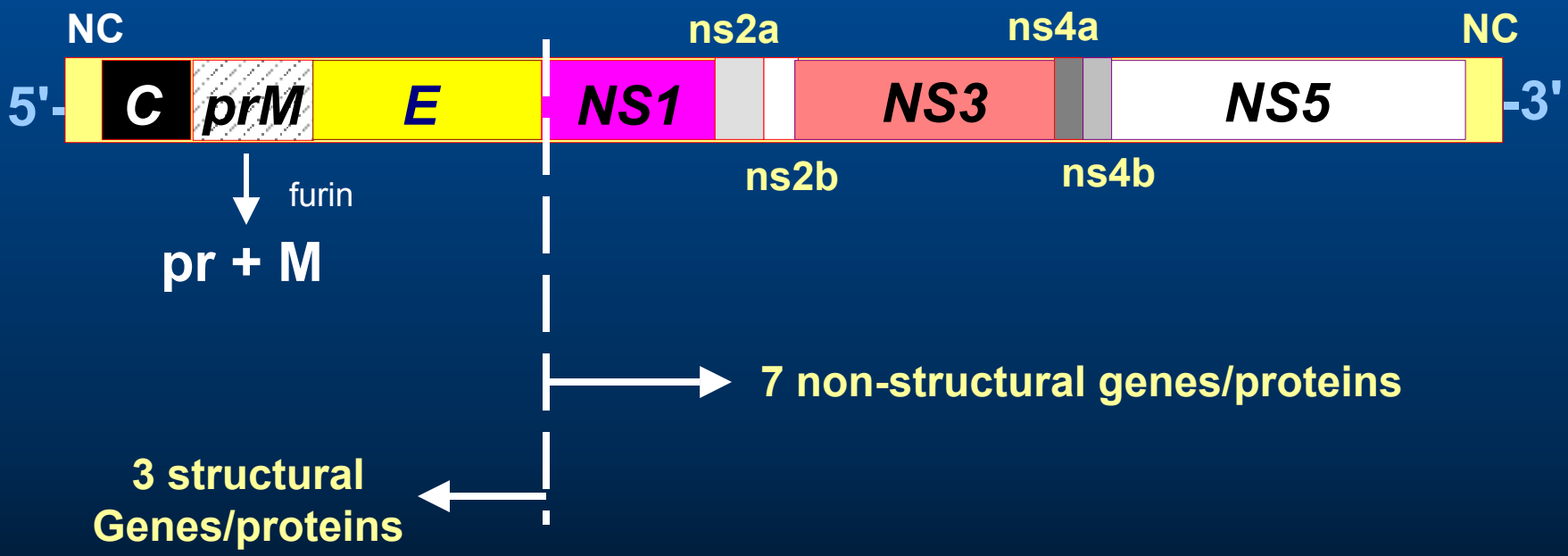
Complete Serological Analysis

		ELISA		PRNT			
Patient	Davs P.I.	IgM (WN)	IgG (SLE)	WN	SLE	DEN2	JE
CSF	8	26.91	1.78	nd	nd	nd	nd
S1	9	9.1	4.16	160	20	<10	10
S2	34	6.7	4.62	1280	20	<10	20
Positive Control	n.a.	9	6.5	>5120	2560	2560	320

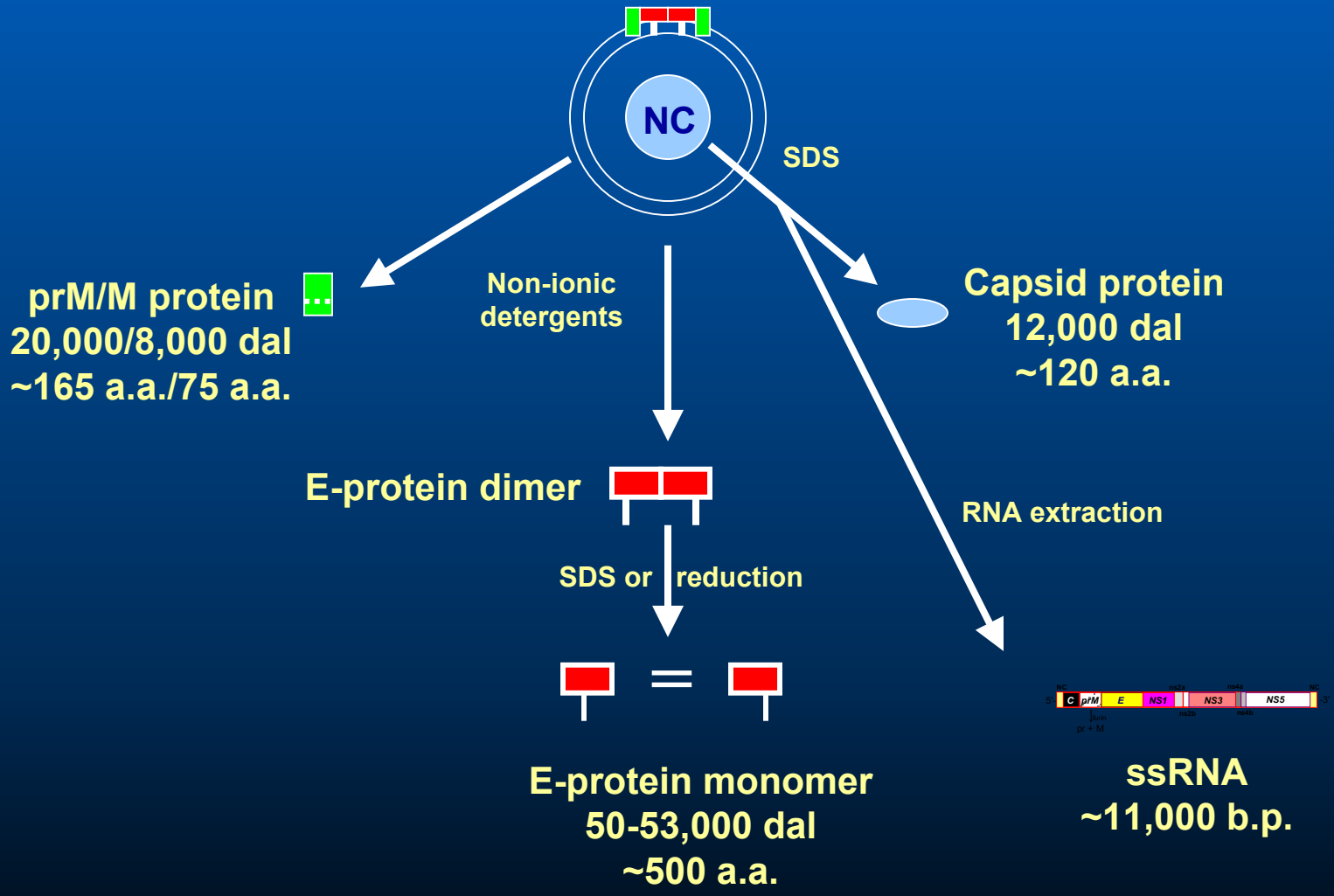
What is the Best Way to Reduce Virus Cross-reactivity?

- Make antigens more specific
- Make antigens less cross-reactive

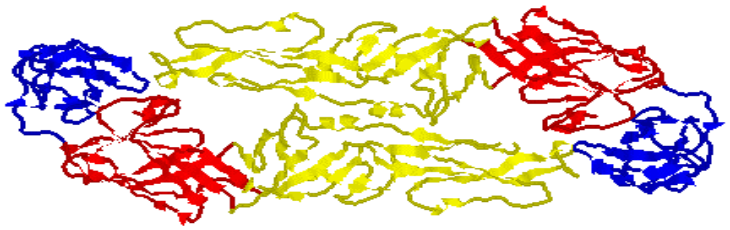
Flavivirus Genome



Virion Components

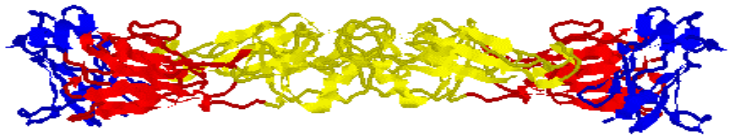


Homodimer of the Flavivirus E-protein



2 Å

Top view



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Lateral view

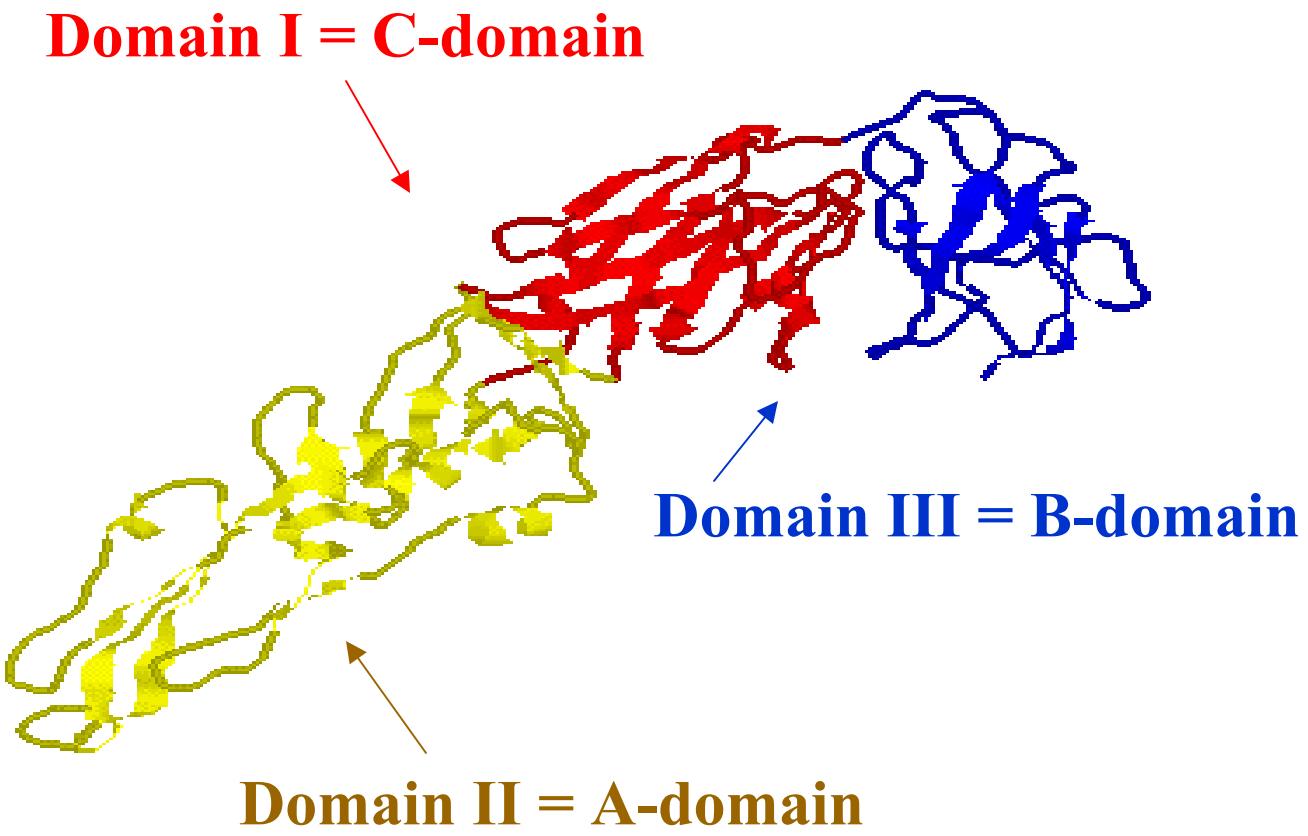
General Antigenic Properties of Flaviviral Proteins - 1

- E-glycoprotein
  - Most important flaviviral antigen
  - Very immunogenic
  - Most serological assays detect reactivity with this protein
- Capsid
  - Elicits primarily group-reactive antibody
- M-protein
  - Very small (75 a.a.)
  - Embedded in the virion envelope membrane and not highly immunogenic

General Antigenic Properties of Flaviviral Proteins - 2

- NS1
  - Cell surface protein
  - Secreted from cells and can be detected in serum samples during some flaviviral infections (*e.g.*, dengue)
  - Very immunogenic
  - Can be used to differentiate infection from vaccination
  - Has some intrinsic viral specificity
- NS3 and NS5
  - Intracellular proteins, antigenic but not very immunogenic
  - NS5 antibody is somewhat virus-specific
  - NS3 antibody is cross-reactive

Domains of the E-protein Monomer



Serologic Cross-reactivities vs.Virus Neutralization Activity of Dengue E-protein Epitopes

Domain	Epitope	Specificity	PRNT
II	A1	Group	+
	A2	Subcomplex	+
	A3	Type	-
	A4	Type	+
	A5	Subgroup	+
III	B1	Type	+
	B2	Type	+
	B3	Subcomplex	-
	B4	Subcomplex	-
I	C1	Subcomplex	-
	C2	Type	-
	C3	Type	-
	C4	Type	-

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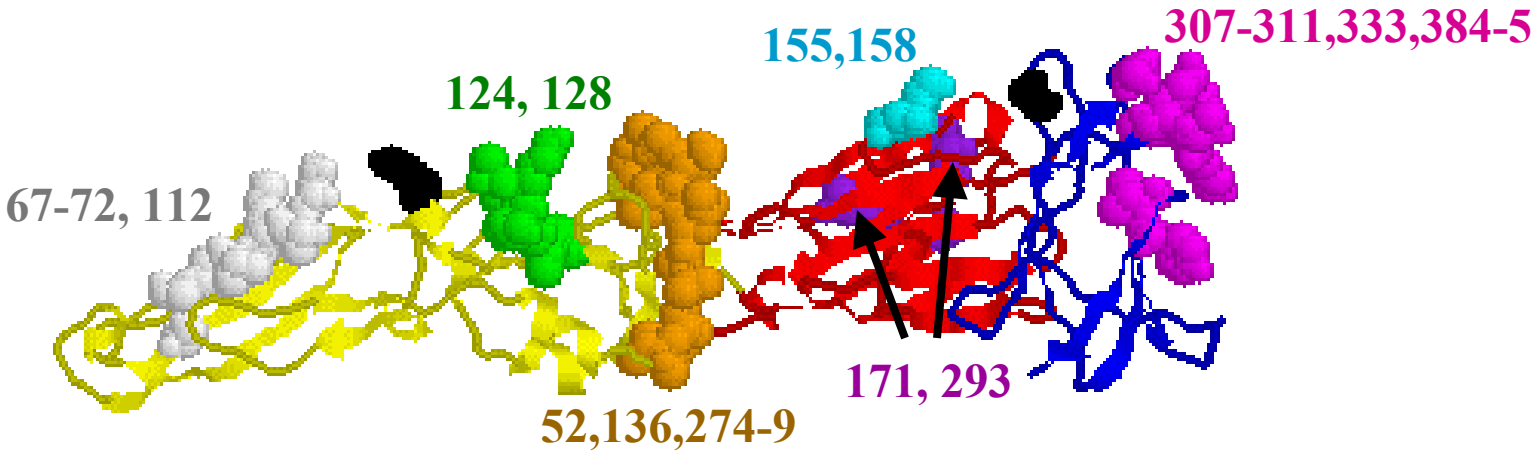
Specificity and Abundance of Neutralizing Epitopes

Virus	Selecting Mab specificity	References
DEN	6 - Type 2 - Subcomplex	Lin 1994, Beasley 2001, Lok 2001
JE	5 –Type 1 - Subgroup	Hasegawa 1992, Cecelia 1991, Morita 2001
LI	3 - Subtype	Jiang 1993, Gao 1994
MVE	5 - Type	McMinn 1995
TBE	5 – Subtype 1 - Complex	Mandl 1989
YF	7 - Type	Lobigs 1987, Ryman 1997
Totals	31 – Subtype or Type 4 – Others	

Location of Neutralization Domains on the Flavivirus E-protein



Pitfalls of Modifying/Expressing the flavivirus E-protein

- Proper expression (conformation) of the E-protein requires co-expression of the prM protein when derived from acidic environments and requires glycosylation
- Domain I and Domain II epitopes are highly conformational; difficult to express on peptides or other small fragments
- Domain III has an essential S-S-bond for epitope expression
- Regions of sequence homology and uniqueness are interspersed throughout the protein.

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Summary

- Increase specificity?
- Increase sensitivity?
- Reduce sample size?
- Increase speed of testing?
- Multiplex?