Mapping the Sequence Space of ZIKA and Related Viruses: High-Content Peptide Libraries for Immune Monitoring

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Introduction: Flaviviridae are a virus family with multiple members threatening human and animal health. Several Flaviviridae are transmitted by arthropod vectors (i.e. ticks and mosquitoes). Mosquito-borne representatives are Yellow fever virus (YFV), Saint Louis encephalitis virus (SLEV), West Nile virus (WNV), Dengue virus (DV), and ZIKA virus (ZIKAV). Although, vaccines were approved for YFV & DV, none are available for other mosquito-borne viruses. Vaccine development is highly dependent on efficient tools for diagnosis and immune monitoring. In light of sequence similarities between viruses and sequence diversity within single virus species differential detection of immune responses at high sensitivity and specificity remains a challenge. We developed an efficient workflow to generate peptide libraries for optimized coverage of this sequence diversity and present resulting peptides libraries in formats that can be easily applied in standardized assay formats for profiling of humoral and cellular immune responses. We describe the design of such ULTRA peptide libraries for ZIKA and related flaviviruses using bioinformatic algorithms, high-throughput chemical synthesis, and peptide presentation in form of antigen spanning ULTRA PepMix™ Peptide Pools for antigen specific T-cell stimulation as well as high-content PepStar™ Peptide Microarrays for profiling of associated humoral immune responses.

Input Sequences

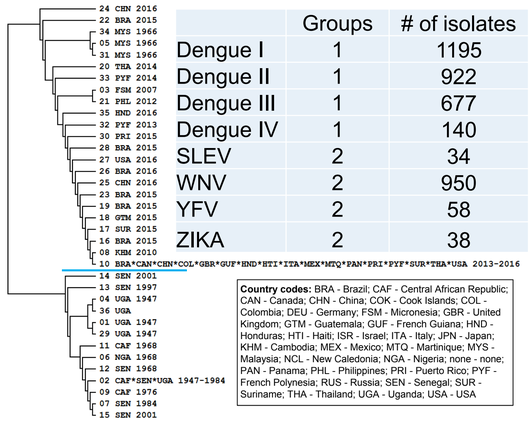
- Polyprotein sequences for ZIKAV, Dengue Virus, SLEV, WNV, and YFV from NCBI. Chikungunya virus (CHIKV) added as control.
- Virtual processing of polyprotein sequences.
- PepStar™- Peptide Microarray library covers structural proteins Capsid Protein (C), Peptide pr (pr), Small Envelope Protein (M), Envelope Protein (E), Non-structural Proteins NS1, NS2A, NS3 and NS5
- PepMix™ Peptide Pool libraries cover proteins C, E, M and NS1 of ZIKAV.

Sequence Diversity in Input Sequences

- High sequence identity in proteins within viruses (**Tab. 1**)
- Lowest conservation in Capsid Protein for Dengue serotype I (86.2 % sequence identity).

	C	pr	M	E	NS1	NS2A	NS3	NS5
Dengue I	86.2	96.1	95.7	97.8	97.6	95.1	98.4	98.2
Dengue II	95.6	93.6	94.2	97.4	96.8	94.9	98.0	97.2
Dengue III	86.4	96.1	96.6	98.0	98.2	97.1	98.8	98.4
Dengue IV	94.8	96.7	96.4	97.7	96.8	94.9	98.6	98.0
SLEV	99.0	98.1	99.3	98.9	97.8	98.7	98.6	97.7
WNV	97.6	98.9	99.0	98.8	98.4	97.9	98.8	98.9
YFV	95.8	98.2	97.7	96.8	97.2	95.3	98.3	96.5
ZIKA	96.3	95.3	96.5	97.7	98.3	97.3	98.6	97.6
Group	48.8	48.3	44.3	53.4	56.5	30.3	64.8	67.6

Tab. 1 Mean sequence identity in percent in protein groups for individual viruses. The last line represents average sequence identity for an alignment of the representative sequences of each virus and subtype.



	Groups	# of isolates
Dengue I	1	1195
Dengue II	1	922
Dengue III	1	677
Dengue IV	1	140
SLEV	2	34
WNV	2	950
YFV	2	58
ZIKA	2	38

Country codes: BRA - Brazil; CAF - Central African Republic; CAN - Canada; CHN - China; COK - Cook Islands; COL - Colombia; DEU - Germany; FSM - Micronesia; GBR - United Kingdom; GTM - Guatemala; GUF - French Guiana; HND - Honduras; HTI - Haiti; ISR - Israel; ITA - Italy; JPN - Japan; KHM - Cambodia; MEX - Mexico; MTQ - Martinique; MYS - Malaysia; NCL - New Caledonia; NGA - Nigeria; none - none; PAN - Panama; PHL - Philippines; PRI - Puerto Rico; PYF - French Polynesia; RUS - Russia; SEN - Senegal; SUR - Suriname; THA - Thailand; UGA - Uganda; USA - USA

Fig. 1. Phylogenetic tree for ENV of ZIKAV. Country codes and year of sample are given. The separation of the sequences into two groups is indicated by the blue line (top: Oceanian/American lineage, bottom: African lineage) . For each group an individual starting sequence for library design was selected. The number of sequence groups for each virus and the number of different isolates used is indicated in the table.

Library Generation

- Sequence diversity in a high number of isolates (table in **Fig. 1**) required pre-selection of protein sequences for optimal coverage
- Based on phylogenetic alignments for each virus/serotype groups were defined (shown exemplary in **Fig. 1**)
- Consensus sequences (CS) for each group were calculated and sequences with highest similarity to CS selected
- Sequences for CHIKV structural proteins capsid, p62, E3, E2, 6k and E1 were added
- Algorithm selected 6256 peptides for PepStar™ Peptide Microrrays (coverage shown in **Tab. 2** and **Fig. 2 and 3**.)
- For PepMix™ Peptide Pools 409 peptides of ZIKA C (48), M (49), NS1 (166), and E (146) were generated for coverage see **Tab. 2**).
- Peptides for peptide microarrays & peptide pools were assembled by high-throughput synthesis and purification.

Products & Applications

- ULTRA PepMix™ Peptide Pools are available for ZIKA C , M, NS1 , and E for T-cell stimulation (e.g. ELISpot, ICS, flow assays)
- A high content ULTRA PepStar™ Peptide Microarray covering all viruses and proteins depicted in **Tab. 1** with 6256 peptides is available for deep and differential profiling of the humoral immune response in humans and animal models.
- The microarrays were used in a recent study for the comparison of the breadth of antibody response for different ZIKA vaccines in rhesus monkeys [1].
- ZIKA-specific immune response after vaccination was assessed using PepMix™-peptide pools in animal studies in mice [2] and rhesus monkeys [1].

References:

1. Abbink P et al. (2016) Protective efficacy of multiple vaccine platforms against Zika virus challenge in rhesus monkeys. *Science*. DOI: 10.1126/science.aah6157.
2. Larocca RA et al. (2016) Vaccine protection against Zika virus from Brazil. *Nature*. DOI: 10.1038/nature18952.

Antigens	C	pr	M	E	NS1	NS2A	NS3	NS5
DENGUE_TYPE_I	82.96	84.74	85.18	90.06	92.4	79.38	94.14	94.52
DENGUE_TYPE_II	81.47	74.18	74.82	90.22	88.7	76.79	91.35	91.3
DENGUE_TYPE_III	88.54	87.91	90.1	92.6	93.1	91.36	96.78	94.23
DENGUE_TYPE_IV	87.96	90.35	86.67	93.19	86.75	78.35	95.78	91.55
YFV	95.32	96.81	97.92	94.53	96.11	88.9	97.34	94.85
ZIKA	95.87	96.7	95.17	98.2	98.75	97.18	99.2	98.12
SLEV	96.11	94.25	98.82	97.62	95.7	96.78	96.71	96.91
WNV	98.7	98.34	98.59	98.89	97.33	98.29	99.37	99.2
ZIKA (PepMix™)	99.9		97.2	94.5	99.1			

Tab. 2. Percent coverage for all proteins in the final peptide libraries.

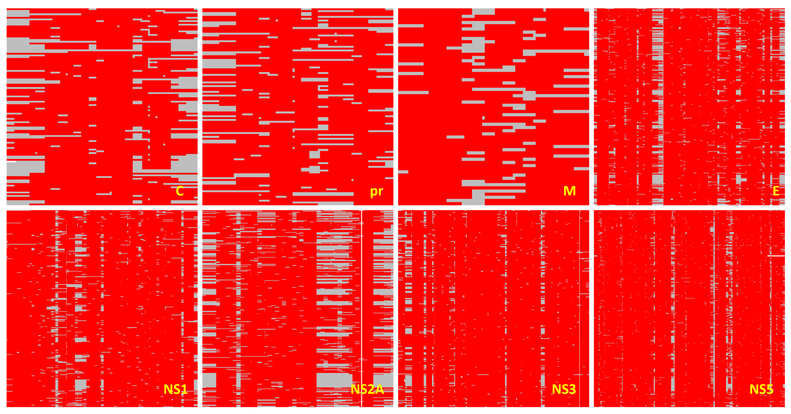


Fig. 2. Sequence coverage for Dengue virus serotype I. Each plot represents one target protein. Each row in the matrix plot represents one of the aligned input sequence of the respective protein with red color indicating sequence stretches covered by our library, grey areas sequences which are not covered and white areas represent gaps in the alignment.

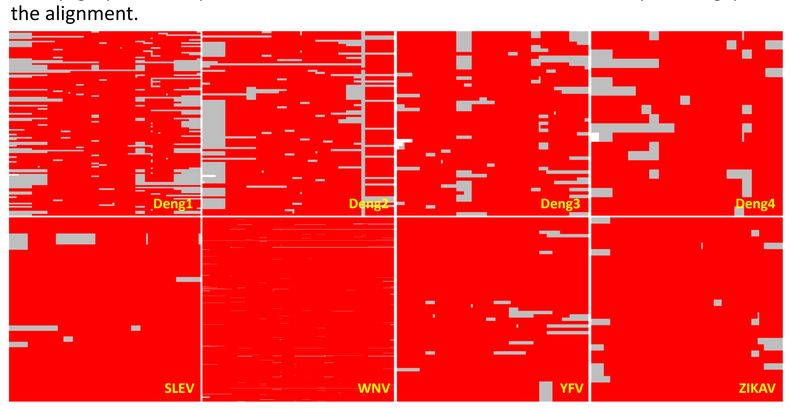


Fig. 3. Sequence coverage for Capsid protein of all covered Flaviviridae (DV serotypes top row, SLEV, WNV, YFV & ZIKAV in bottom row). The color code is according to Fig. 2.

- **Comprehensive peptide libraries were designed for deep immune profiling of ZIKA & other flaviviruses.**
- **PepStar™ Peptide Arrays allow deep profiling of B-cell immune response with minimum serum (1µl/assay).**
- **PepMix™ Peptide Pools enable effective antigen specific T-cell stimulation .**
- **ZIKA PepStar™ Peptide Arrays and PepMix™ Peptide Pools applied for monitoring immune responses stimulated by several candidate vaccines [1,2].**

Acknowledgment

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