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# The flavivirus capsid protein: Structure, function and perspectives towards drug design

Edson R.A. Oliveira[a,*], Ronaldo Mohana-Borges[b], Ricardo B. de Alencastro[a], Bruno A.C. Horta[a]

[a] Laboratório de Modelagem Molecular, Instituto de Química Orgânica, Universidade Federal do Rio de Janeiro, RJ, Brazil
[b] Laboratório de Genômica Estrutural, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

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ABSTRACT

Flaviviruses, such as dengue and zika viruses, are etiologic agents transmitted to humans mainly by arthropods and are of great epidemiological interest. The flavivirus capsid protein is a structural element required for the viral nucleocapsid assembly that presents the classical function of sheltering the viral genome. After decades of research, many reports have shown its different functionalities and influence over cell normal functioning. The subcellular distribution of this protein, which involves accumulation around lipid droplets and nuclear localization, also corroborates with its multi-functional characteristic. As flavivirus diseases are still in need of global control and in view of the possible key functionalities that the capsid protein promotes over flavivirus biology, novel considerations arise towards anti-flavivirus drug research. This review covers the main aspects concerning structural and functional features of the flavivirus C protein, ultimately, highlighting prospects in drug discovery based on this viral target.


**Contents**



## 1. The growing need for anti-flavivirus drugs

Flaviviruses comprise over 70 small enveloped viruses that are members of the *Flavivirus* genus of the *Flaviviridae* family (Lindenbach and Rice, 2003). Despite the numerous existing flaviviruses, only a small fraction is clinically relevant to humans with transmissions predominantly mediated by arthropods, such as mosquitoes or ticks. *Aedes* mosquitoes are recognized as the most significant vectors and are distributed among tropical and subtropical regions of the globe. Flavivirus diseases with global health implications are in general caused by West Nile (WNV), yellow fever (YFV), japanese encephalitis (JEV), tick-borne encephalytis (TBEV), dengue (DENV) or zika (ZIKV) viruses (Gubler et al., 2007; Weaver et al., 2016). WNV was first described as a human pathogen in 1937 and ever since the occurrence of West Nile fever has been reported in many parts of the world (Smithburn et al., 1940; Sejvar, 2016). Yellow fever and Japanese encephalitis are also still found as important diseases mainly in Africa and Asia, respectively (Wang and Liang, 2015; Butler, 2016). In Europe, TBEV is considered as one of the major contributors for central nervous system (CNS) infections and also represents a relevant threat in Asia (Kaiser, 2016). Additionally, diseases caused by DENV and ZIKV have been recently highlighted due to their critical impact over quality of life. DENV transmission has raised as a serious threat in

* Corresponding author at: Av. Athos da Silveira Ramos, 149 – Centro de Tecnologia, Bloco A, Sala 609, CEP: 21941-909, Instituto de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil.
*E-mail address:* edsonrao@gmail.com (E.R.A. Oliveira).

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view of the relevant international prevalence (Bhatt et al., 2013; Guzman and Harris, 2015) and ZIKV has been associated to severe life-long sequels (Araujo et al., 2016).

Among the flavivirus diseases, dengue is considered the most prominent one. It yields an estimated 390 million infections with nearly 20,000 casualties every year worldwide (Bhatt et al., 2013; Guzman and Harris, 2015). As dengue occurs in underdeveloped and developing areas, a peculiar feature during outbreaks is the large need for medical care. This causes an overflow of public health units, which invariably worsens ongoing crisis (Lowe, 2015). Another important aspect regarding dengue is the considerable number of different attempts in vaccine research. Since DENV exists as four distinct serotypes (DENV1–DENV4), a desired vaccine should be able to induce protective immunity against all of them, simultaneously. Due to the high degree of similarity among DENV serotypes, interference between elicited immune responses may happen, which makes the vaccine developing process extremely laborious and challenging (Thomas and Rothman, 2015). Fortunately, a tetravalent live-attenuated anti-DENV chimeric vaccine has recently become available for commercial purposes (Durbin, 2016). This fresh and eye-catching fact increased hope for better quality of life in affected areas, as well as curiosity about the post-marketing surveillance of this new vaccine.

Special attention is now attributed to ZIKV transmission due to its recent considerable spread in the Americas (Faria et al., 2016), and also because infections by this virus have been linked to severe neurological complications such as microcephaly (Araujo et al., 2016; Petersen et al., 2016a; Garcez et al., 2016) and Guillain-Barré syndrome (Araujo et al., 2016; Watrin et al., 2016; Malkki, 2016). Only in Brazil, the accumulated number of infections has been estimated from 440,000 up to 1.300,000 (Bogoch et al., 2016), with microcephaly prevalence of nearly 100 cases per 100,000 livebirths (Ventura et al., 2016). Still in the epidemiological outlook, affected countries can attract visitors due to their tropical climate, business opportunities or even by the occurrence of mass-gathering events (Petersen et al., 2016b). Non-affected areas that potentially attract people from places where transmissions occur are also at risk. That is the case of Saudi Arabia where populated pilgrim events occur (*e.g.* The Umrah and Hajj) (Elachola et al., 2016). Hence, a possible spread of ZIKV to other regions where no transmissions are reported is considered an ongoing risk (Lessler et al., 2016). A recent study about ZIKV suggested that the vaccine development against this virus is probably enrolled with a favorable pipeline (Larocca et al., 2016). Still, in practical terms, such countermeasure remains as a highly desired research goal.

Considering other flaviviruses against which human vaccines have been available for decades, *e.g.* YFV (Verma et al., 2014), JEV (Yun and Lee, 2014) and TBEV (Steffen, 2016), high incidence levels are still reported in major affected areas (Campbell et al., 2011; Vasconcelos and Monath, 2016; Kunze, 2016). Yellow fever poses a threat mainly to Africa while JEV is still transmitted in Asia and in Western Pacific regions, with global death numbers of about 30,000 (Lucey and Gostin, 2016) and 17,000 (Campbell et al., 2011) every year, respectively. TBEV is another menace that is found relevant in the European Union and in the European Economic Area, where about 50 million travelers are received per year (Kunze, 2008). Russia is also affected by up to 10,000 cases annually (Kaiser, 2012). Altogether, even classical flavivirus diseases that can be prevented by active immunizations still yield relatively high incidence levels.

In view of all the above points, the overall scenario claims for a better global control of flavivirus diseases. In addition to vaccination, actions such as vector management, educational programs and antiviral development would represent valuable options. The latter is specially precious because antivirals are specific, provide short-term effects and can be administered according to necessity, increasing professional management. In the particular case of ZIKV infections, antivirals could also be used in order to avoid (or minimize) fetal neurological disorders. Over the last decades, only limited efforts and capital have been invested in anti-flavivirus drug design. As a consequence, not a single anti-flavivirus drug candidate has been selected from clinical trials. This is extremely unfortunate given the relevance of flavivirus diseases for public health. Nevertheless, the recent menace provided by ZIKV has led to increased number of studies in this sense. An *in-vitro* screening considering 774 FDA-approved drugs was carried out revealing that more than 20 compounds are active against ZIKV infection (Barrows et al., 2016). This encouraging result is an indicative of possible novel drug candidates for future anti-ZIKV studies.

## 2. Why targeting the capsid protein?

Several anti-flavivirus approaches consider different molecular targets including host and viral elements (Noble et al., 2010; Beesetti et al., 2016; Kok, 2016). By using drug candidates that modulate host factors, the likelihood of interference in normal physiological functions is increased. For instance, the usage of corticosteroids or statins to prevent the progress of dengue disease was related to adverse effects such as hyperglycemia (Tam et al., 2012), hepatic dysfunction, thrombocytopenia and mucosal bleeding (Whitehorn et al., 2012, 2016). Alternatively, aiming at viral elements can be generally considered as a safer strategy, minimizing possible interferences in host homeostasis.

During infection, the flavivirus genome is translated into a large polyprotein that is further enzymatically processed to originate ten viral proteins: three structural (pre-membrane – prM, capsid – C and envelope – E) and seven non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) (Lindenbach and Rice, 2003). In principle, each of them could be considered as target for antiviral intervention. The E protein is the main structural component present in the virus particle and participates in key steps during the viral life cycle, such as virus binding and entry in susceptible cells. NS3 and NS5 are enzymatic targets with also well described structures and functions (Lindenbach and Rice, 2003). Several drug-design studies have been carried out by independent research groups with the hope of finding small inhibitor compounds, considering these three referred proteins: E (Modis et al., 2003; Zhou et al., 2008; Wang et al., 2009; Kampmann et al., 2009; Poh et al., 2009; Alen and Schols, 2012), NS3 (Tomlinson et al., 2009a, 2009b; Bodenreider et al., 2009; Yang et al., 2011; Tomlinson and Watowich, 2011, 2012; Cregar-Hernandez et al., 2011; Mastrangelo et al., 2012; Byrd et al., 2013a) and NS5 (Yin et al., 2009a, 2009b; Niyomrattanakit et al., 2010, 2011; Lim et al., 2011; Nguyen et al., 2013; Noble et al., 2013). Additional approaches also targeted the NS4B protein (van Cleef et al., 2013) and some celular elements, such as glucosidases (Watanabe et al., 2012), kinases (de Wispelaere et al., 2013) and factors involved with host immunity (Castro et al., 2011; Salgado et al., 2012). Unfortunately, these attempts have led to compounds showing common limitations of the drug-design pipeline, such as poor selectivity, undesired pharmacokinetic properties, poor stability, poor permeability, inactivity in cell-based assays, low efficacy, among others.

Given this challenging scenario, the consideration of additional viral targets could be a promising alternative for anti-flavivirus drug development. In this context, the C protein is considered an interesting target because it plays a crucial role in flavivirus biology. During the virus life cycle, a collection of C protein subunits are assembled to form nucleocapsids, that are known to encase the genetic material of viral pathogens. In addition to this classical structural role, a growing number of evidences associate the C protein to other important functions in the viral replication cycle. It was found that a cleavage region present in the capsid sequence,