Application note

Rapid identification and characterisation of Zika NS1 specific Affimer® binders

Summary

- Identification and validation of the Zika NS1 Affimer binders was completed within 16 weeks.
- Affimer binders display exquisite specificity amongst the flavivirus family.
- Affimer binders can specifically identify Zika NS1 at clinically relevant concentrations in serum samples.
- Replacing antibodies with Affimer binders improved performance in a commercially available ELISA.

Introduction

Following the emergence of the Zika virus in South America in 2015, the World Health Organisation declared the situation a worldwide public health emergency.

The Zika virus has been causally linked to a rise in the number of cases of microcephaly and brain damage in babies and temporary paralysis from Guillain-Barré syndrome in adults.[1] Due to the homologous nature of the Flaviviridae virus family, to which Zika belongs, the development of diagnostic assays for the Zika virus has been limited, with Zika antigens and antibodies exhibiting cross-reactivity with those of the other Flavivirus family members.[2, 3]

The rapid identification and characterisation of specific, high affinity binders to a wide variety of often challenging targets is a key benefit of Affimer technology. We have utilised this to develop Affimer binders to a recombinant form of the Zika virus non-structural protein 1 (NS1) that is diagnostic of Zika infection at the early, acute phase.[3, 4, 5] Within just sixteen weeks we were able to generate specific Affimer binders to this challenging target (Fig.1), and have further utilised these Affimer binders within an immunoassay format that is able to specifically detect the Zika virus NS1 antigen at clinically relevant concentrations.

Avacta®

Rapidly identifying Affimer binders to Zika

Our diverse Affimer phage library, containing 10^{10} sequences, was screened using a recombinant Zika NS1 target protein of the Suriname strain sourced from the Native Antigen Company (Oxford, UK).[6]

Following subcloning and expression as Affimer proteins, candidate Affimer binders to the Zika NS1 target were identified via a high throughput bead-based direct binding assay that allows the relative binding of different proteins to be analysed and indicates target specificity of the Affimer binders.[7]

Using this strategy, we were able to select several Affimer candidates that showed high affinity binding to the Zika NS1 protein, with limited affinity for eight other highly homologous viral NS1 proteins tested (from Dengue, Yellow Fever, West Nile Japanese Encephalitis and Tick Born Encephalitis viruses), within just eight weeks (Fig. 1).

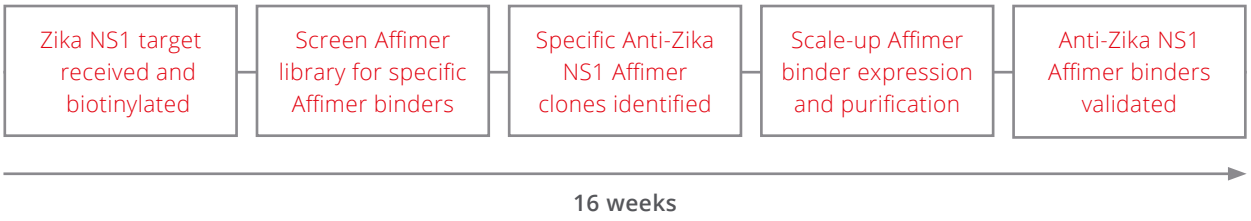

***Figure 1**: Specific Affimer binders to the Zika NS1 protein were identified, expressed, purified and validated within just 16 weeks of receipt of the target material.*

Avoiding cross-reactivity for clinical targets with Affimer binders

Selected Affimer binders were subject to an Octet® binding assay in order to assess their target affinity and specificity (Fig. 2).

The anti-Zika NS1 Affimer binders exhibited no significant cross reactivity for any of the similar viral NS1 protein homologues. The observed specificity of the Affimer binders for the Zika NS1 protein over other homologous viral NS1 proteins demonstrates the power of the Affimer technology in being able to select for highly selective binders, even amongst very closely related protein targets.

The specificity of the Affimer binder for the Zika NS1 target protein was further exemplified by immunoprecipitation experiments (Fig. 3). Affimer binders were immobilised on beads and incubated with human serum spiked with viral NS1 protein. Examining these samples via Western blot, using an anti-His mouse monoclonal antibody for detection, demonstrated that the anti-Zika Affimer binder is highly specific for the Zika virus, only pulling down the Zika NS1 protein from the serum samples, with no affinity for the homologous NS1 proteins from the other viruses tested.

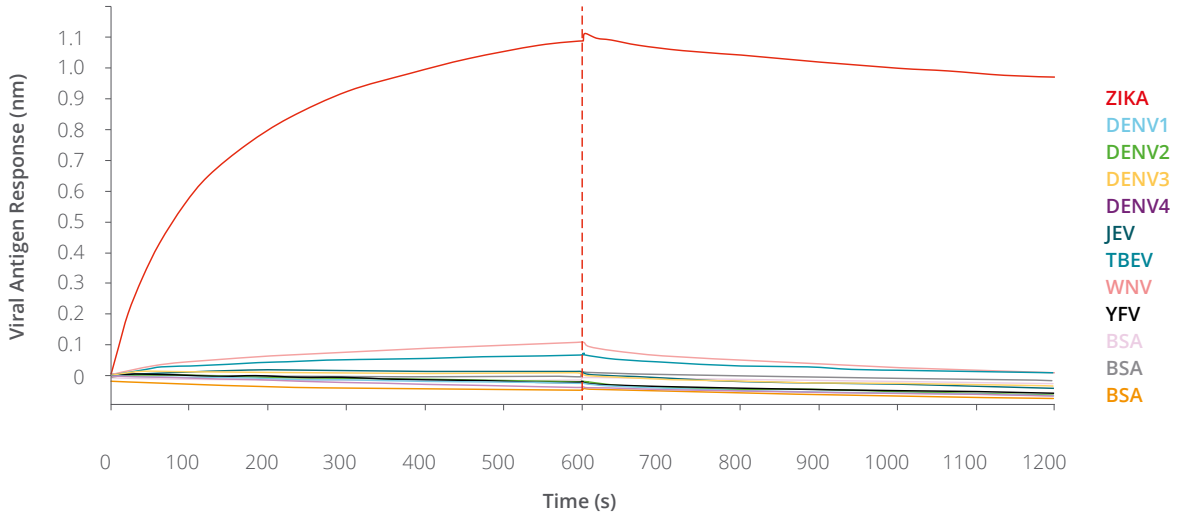

Figure 2: *Octet® analysis of the anti-Zika Affimer binder showed specific binding to Zika NS1 with low binding to the NS1 protein homologues from related viruses, including four variants of the Dengue virus, Japanese Encephalitis Virus, Tick-Borne Encephalitis Virus, West Nile virus and Yellow Fever virus. A representative curve is shown of the binding analysis of the various viral NS1 proteins carried out by biolayer interferometry using an Octet RED system that displays binding as a wavelength shift (in nm) in real time.*

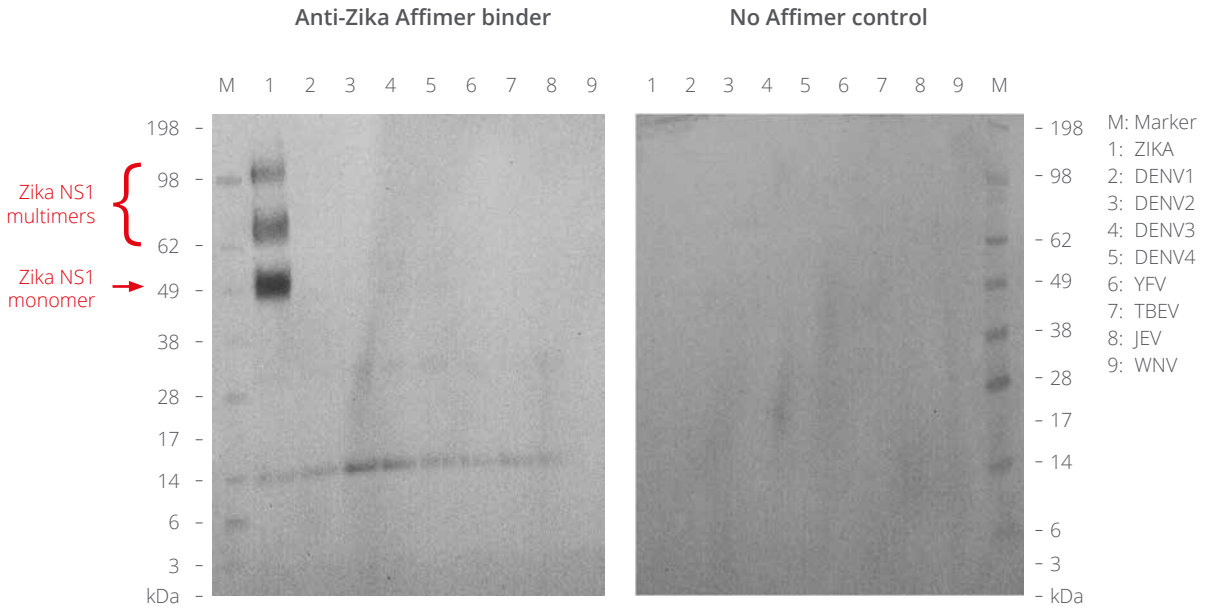

Figure 3: *Affimer protein-coated beads were used in an immunoprecipitation experiment with the Zika NS1 protein and other closely related viral NS1 proteins. The anti-Zika NS1 Affimer binder successfully pulls-down His-tagged Zika NS1 protein from a human serum background, yet shows no affinity for any of the other His-tagged viral NS1 proteins analysed. Samples were run on reducing Western blots and detected with an anti-His mouse monoclonal-HRP conjugate at 1:1000, revealing the Zika NS1 protein present as a monomer and higher molecular weight multimers.[8]*

Affimer - antibody pairs offer increased assay sensitivity

After testing various Affimer plate formatting options, the anti-Zika Affimer binder optimal performance was observed when using an Affimer GST fusion directly adsorbed on Maxisorp® plates, thus this format of the Affimer binder was used for subsequent experiments.

To evaluate the performance of our anti-Zika Affimer binders we carried out a side-by-side comparison with a commercially available ELISA sandwich assay for the detection of the Zika virus NS1 protein. The capture antibodies from the commercially available kit were directly replaced with the anti-Zika Affimer binders and paired with the kit's detection antibody. The Affimer proteins showed improved performance compared to the commercial capture antibody in terms of assay sensitivity, both in buffer and against the complex background of human serum (Fig.4), when performing the assay according to the manufacturers recommended protocol, using our recombinant Zika NS1 antigen. Additionally, replacing the capture antibody of the commercial kit with an anti-Zika NS1 Affimer binder was observed to lower the assay's limit of detection by a factor of three (to a LOD ≈ 140 pg/mL).

This demonstrates that before any assay optimisation, the anti-Zika Affimer binders were able to specifically identify the Zika antigen within a complex sample at clinically relevant concentrations, exemplifying their potential for validation in clinical assays. In this format, screening for Affimer-antibody pairs may offer improved performance for many current and novel immunoassays.

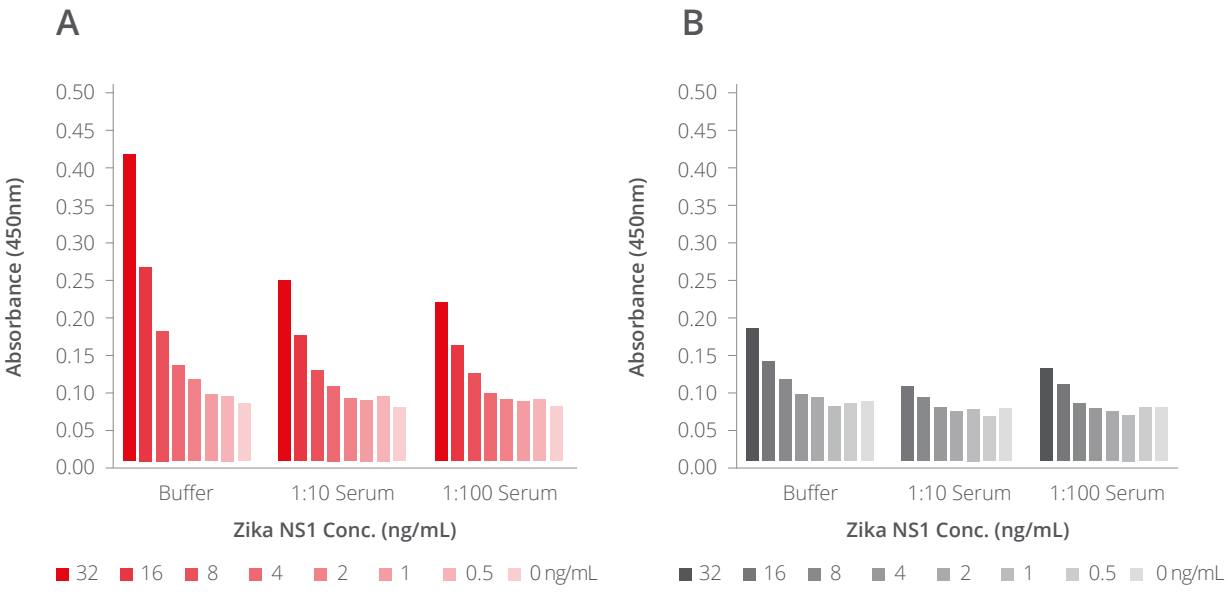

Figure 4: *Affimer binders show comparable performance to a commercially available Zika ELISA detection kit in terms of sensitivity and limit of detection. Manufacturer capture antibodies were replaced with Affimer binders within a commercial Zika sandwich ELISA. Absorbance was measured using the kit-provided and our in-house Zika NS1 antigen in buffer and diluted in serum on both (a) Affimer-coated ELISA plates and (b) manufacturer provided ELISA plates.*

Summary

The ability to rapidly develop highly specific binders to challenging targets, such as the Zika NS1 antigen, meets a previously unmet need in the field of affinity proteins. Beyond reducing project timelines, the potential application of this technology to diagnostic assay development offers advantages in improving the specificity, sensitivity and limit of detection for both current and novel assays.

Here, we were able to apply Affimer technology to identify and characterise Affimer binders to a clinically relevant target within just sixteen weeks.
Using these binders in an Affimer immunoassay system we have demonstrated that they are able to specifically identify the Zika virus NS1 protein antigen at clinically relevant concentrations.[9, 10] Our Affimer binders showed no cross-reactivity to the homologous viral NS1 proteins even against a complex background of human serum. Furthermore, in a side-by-side comparison to an antibody-based commercially available ELISA kit for Zika detection, without any assay optimisation, our Affimer binders showed improved performance to the capture antibody in terms of target sensitivity and limit of detection. Utilising Affimer-antibody pairs in this format could increase assay performance in terms of target specificity and sensitivity for current and novel target assays.

Affimer technology offers an effective platform to develop tools for diagnostic assays being able to increase both the speed of development and assay performance.

References

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Affimer®

Avacta

Unit 20, Ash Way,

Thorp Arch Estate,

Wetherby, LS23 7FA

United Kingdom

Tel: +44 (0) 1904 21 7070

www.avacta.com

@Affimers

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