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Structural characterisation and inhibition of Arenavirus replication complex elements : assembly, function and inhibition of embedded nucleases.

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INTRODUCTION

Arenaviruses, belongs to a family of emerging enveloped segmented and ambisens RNA viruses associated with neurological and hemorrhagic diseases in humans. Arenavirus transcription and genome replication are cytoplasmic ensured by a ribonucleoprotein replicase complex NP-L. After penetration, L protein initiates transcription to produce NP and L mRNAs [1]. The priming of transcription is the result of a *cap-snatching mechanism* ensured by an endonuclease domain associated to the L polymerase. As the concentration of NP in the cell increases, genome segments are replicated, to produce full-length copies (cRNA). cRNAs are now templates for transcription of GPC mRNA (from the S segment) and Z mRNA (from the L segment). The NP carries an *exonuclease* in charge of clearing out from the cytoplasm dsRNA triggering innate immunity response. Both nucleases have a similar *two metal ion catalytic mechanism*, with the particularity of transitioning ion brought by the RNA substrate. Any alteration of the remaining ion impairs greatly these activities[2]. We present a global study aiming to characterize the assembly of the NP[3], through flexible domains[4], a step critical for vRNA packaging and the positioning of L for vRNA replication, as well as using a combined approach of biophysical screening, *crystallography* and *in silico* docking, identifying active compounds against both nucleases[5]. Crystal structures of the nucleases domain complexed with several compounds were obtained[6,7]. By developing specific compounds to alter both transcription and innate immunity shadowing, our strategy is to give the cell a fighting chance to clear the infection. Combining structure, enzymology, rational synthesis, hit-to-lead optimization, *in cellula* evaluation, and screening methods, we are presenting the results of a 2nd generation of molecules paving the way to the design of a 3rd generation increasing specificity towards Arenaviral nucleases in the context of the replication complex[8].

STRATEGY

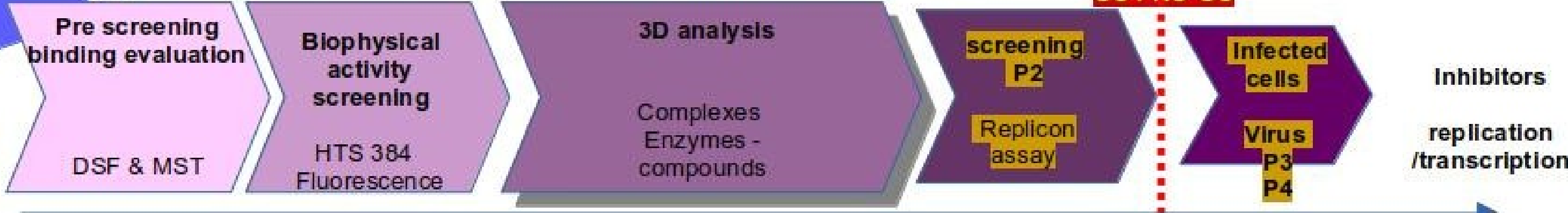
Biochemical characterization

Structural Characterization

Inhibition

Single enzyme domain Or in complex

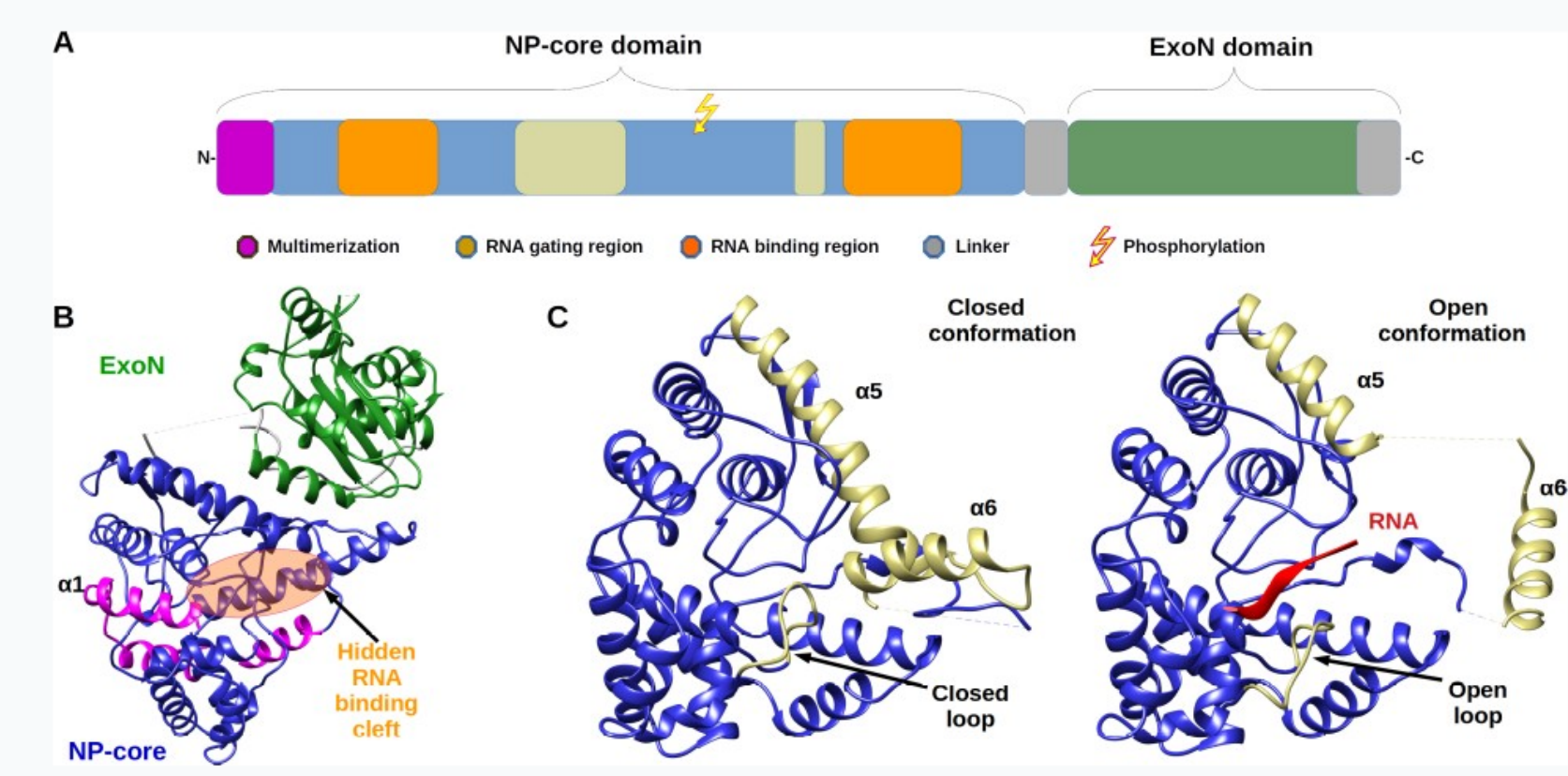
→ Screening pipeline



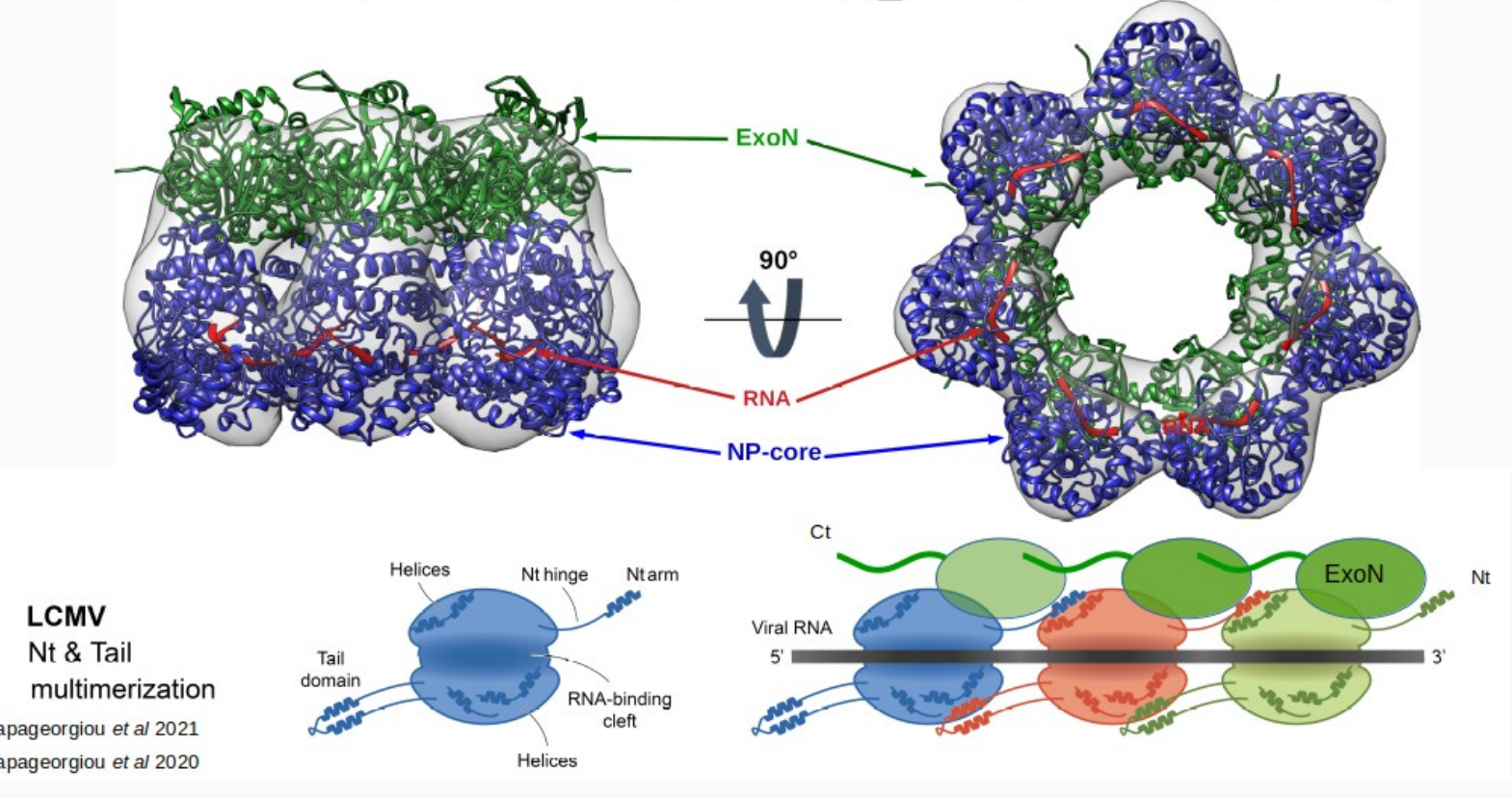
RESULTS

NP characterization

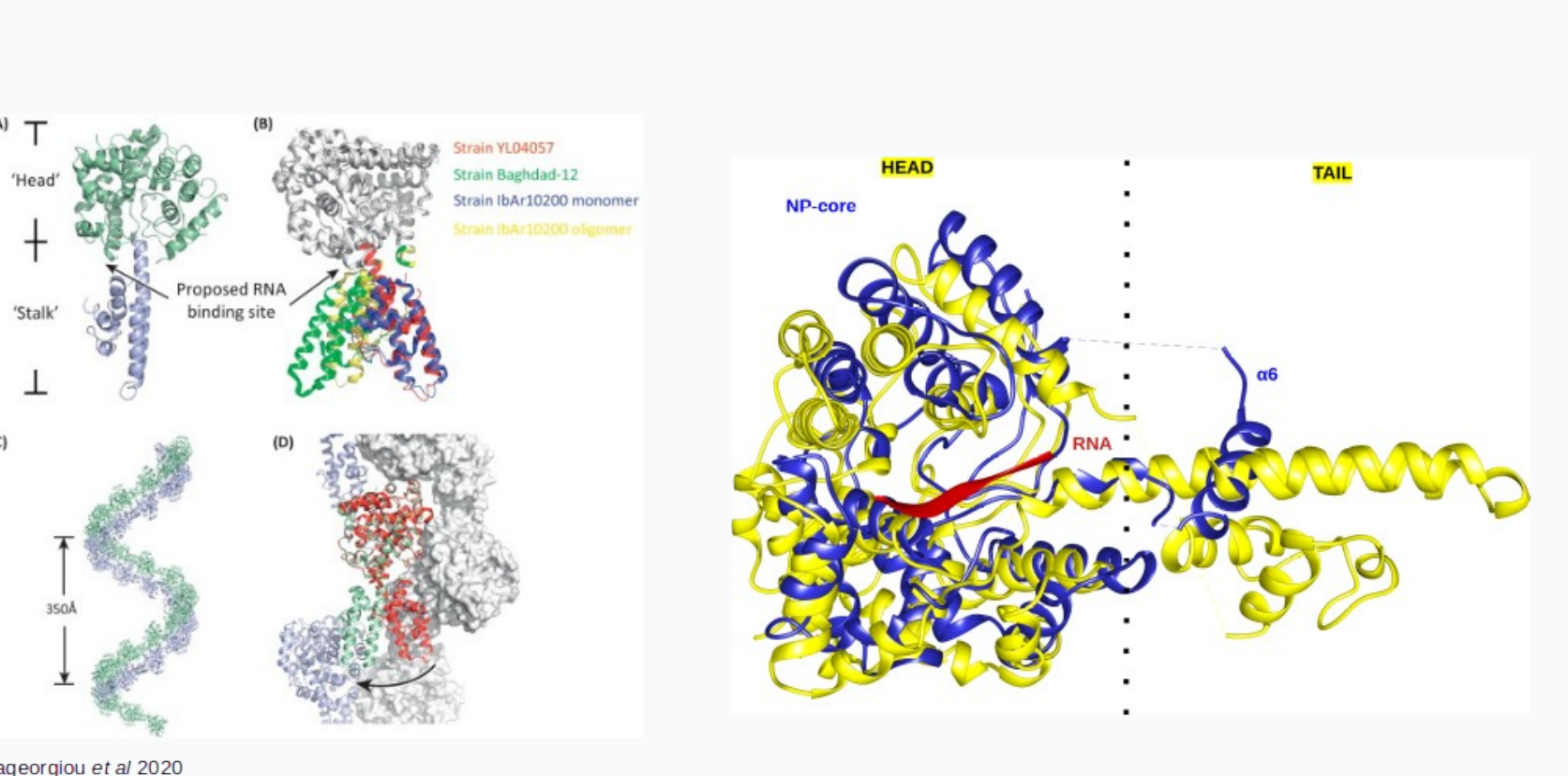
Arenavirus Nucleoprotein Architecture



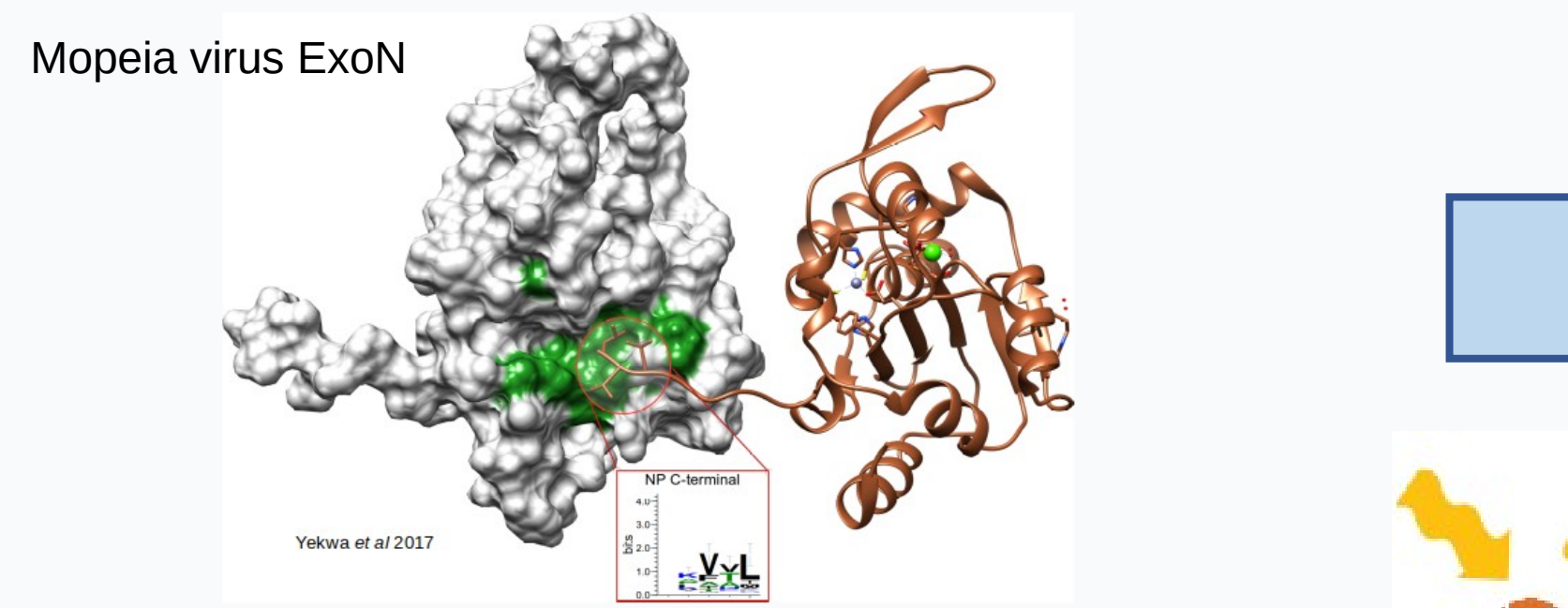
Structural model fitting structural data



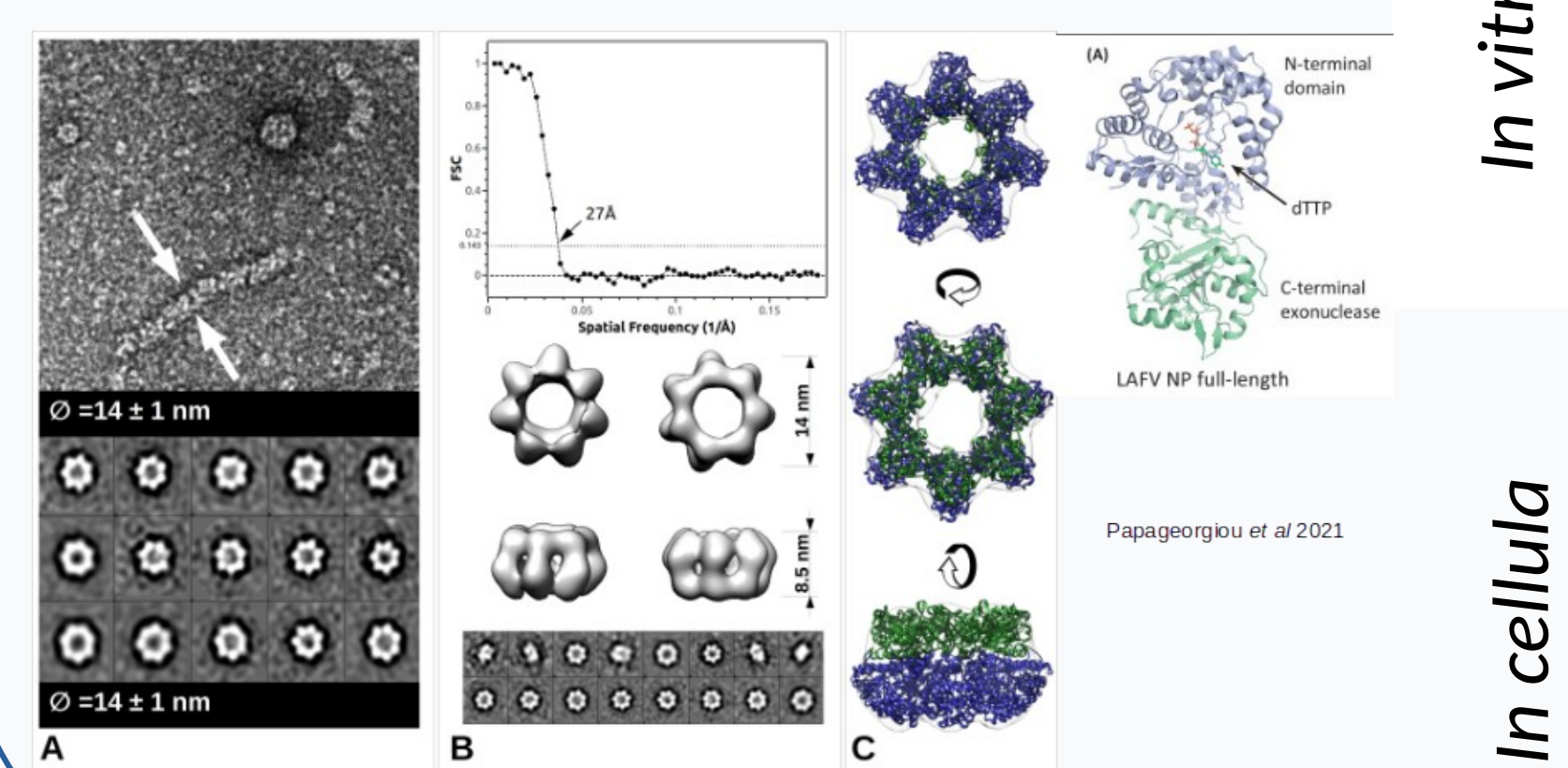
Structural similarities with *Nairovirus*' N



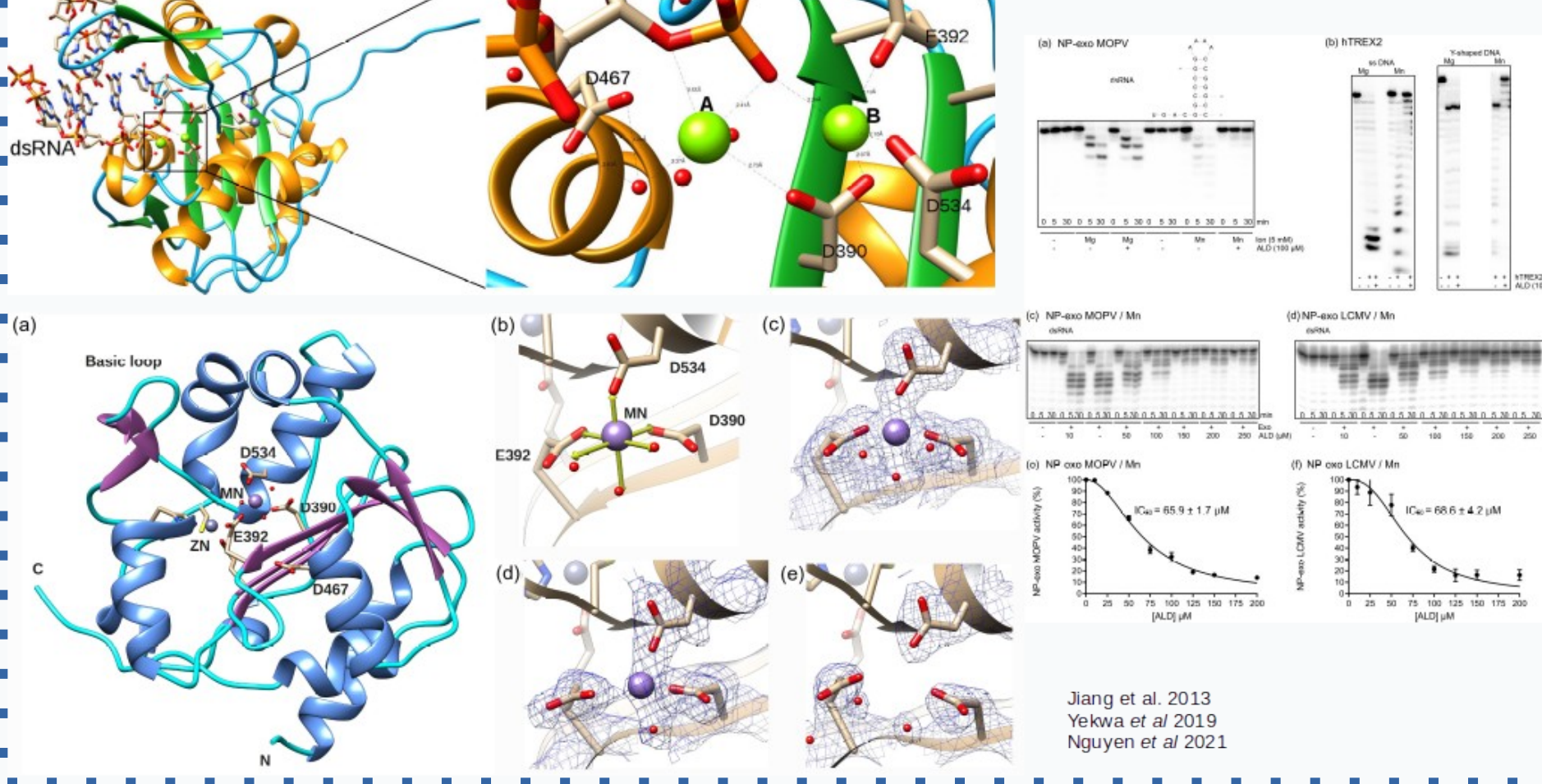
C-terminal ExoN multimerization tail



Arenavirus RNP Reconstruction

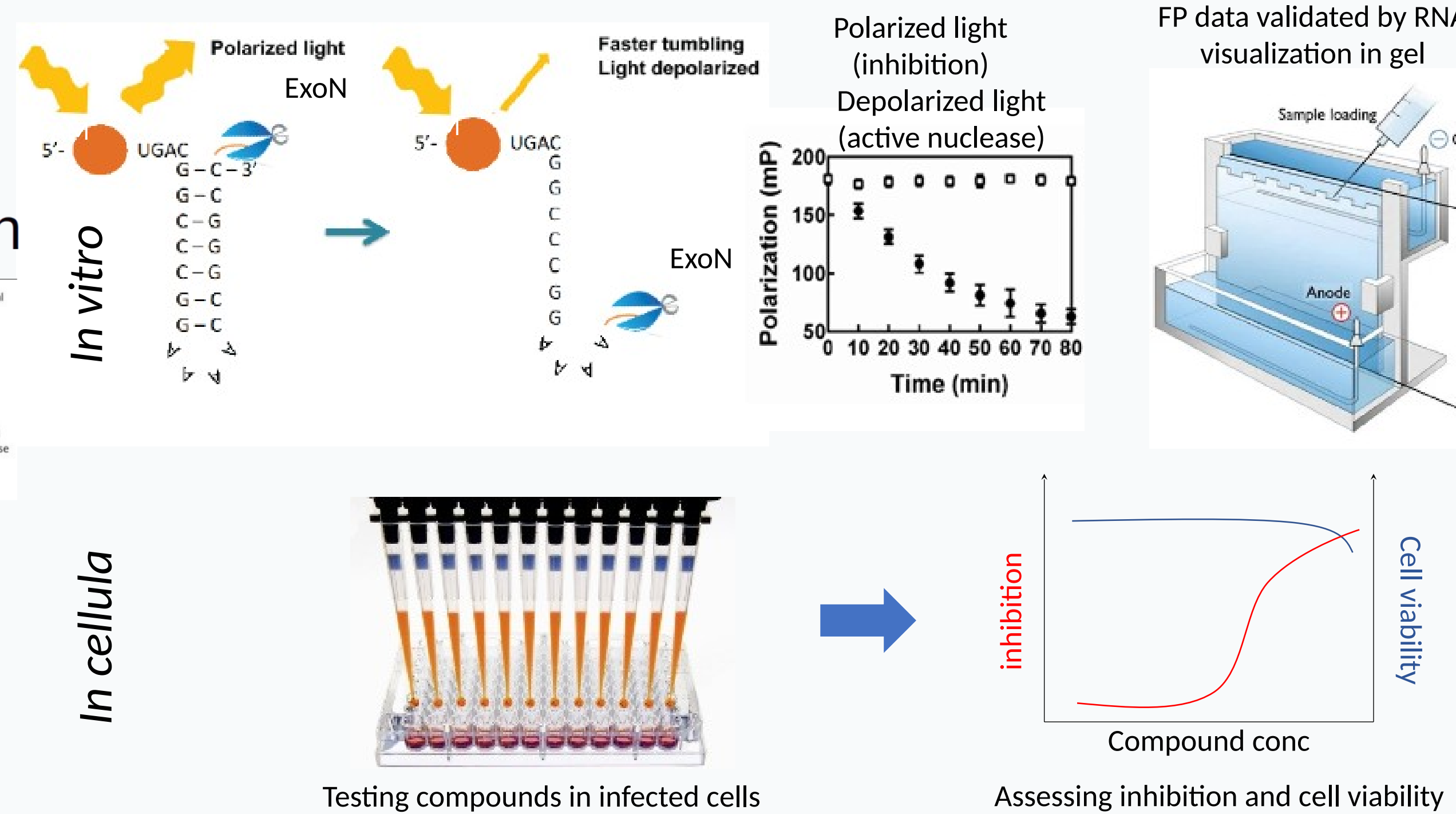


ExoN MOA & Inhibition by ion chelation



Chelation thru time structures : disappearance of the ion in presence of the inhibitor. Biochemical evaluation of the compound.

Screening Methodology



ARENAVIRUSES

Viruses transmitted by rodents causing severe human diseases

Lymphocytic choriomeningitis Virus (LCMV):

- World wide distribution
- Causes choriomeningitis

Lassa Fever:

- Around 300 000 people infected per year
- 6000 deaths / major health problem with severe hear loss / social disruption
- 80% mortality rate for pregnant women

GENOME ORGANIZATION

Segmented negative strand RNA genome

S RNA (3.5 kb)

UTR IGR UTR

5' GPC dN 3'

L RNA (7.2 kb)

UTR IGR UTR

5' 3'

VIRION STRUCTURE

- RNA genome
- RNA polymerase + EndoNuclease
- Nucleoprotein + ExoNuclease
- Matrix protein
- Ribosomes
- Glycoprotein

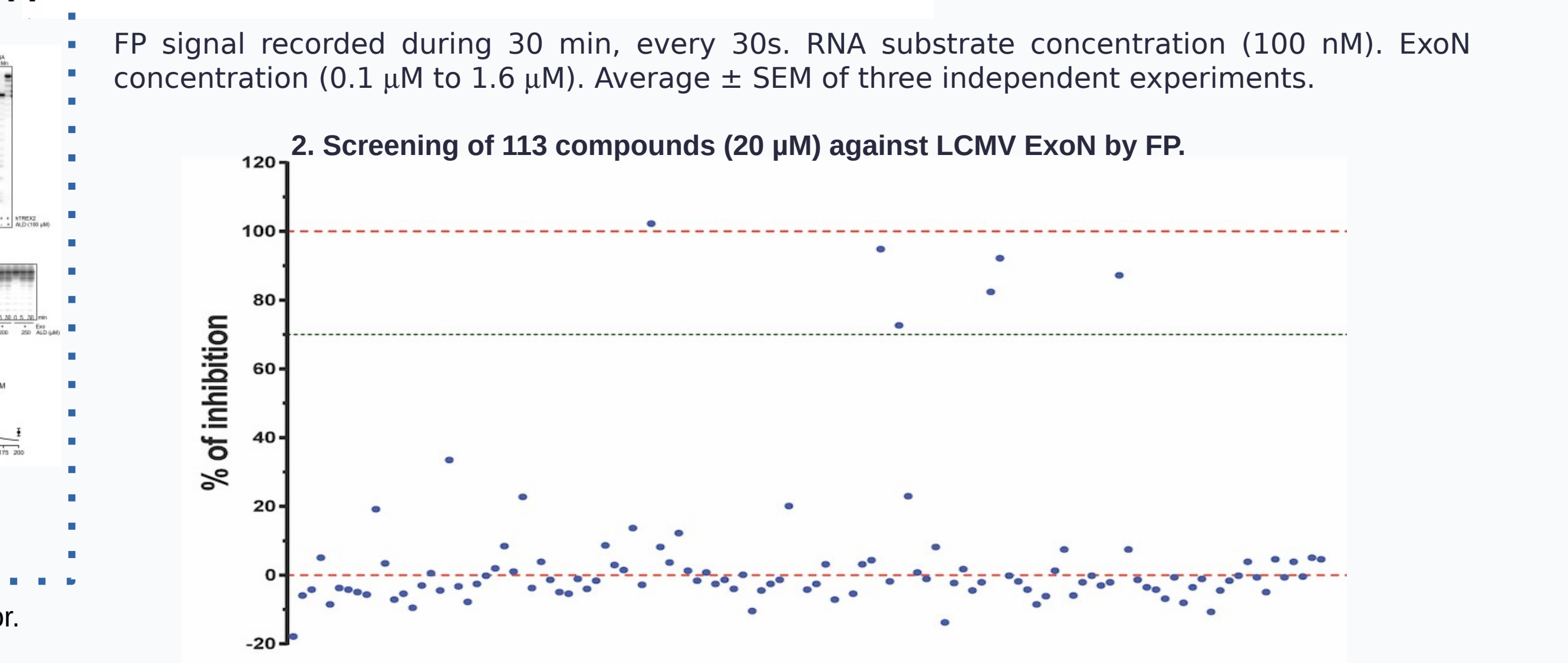
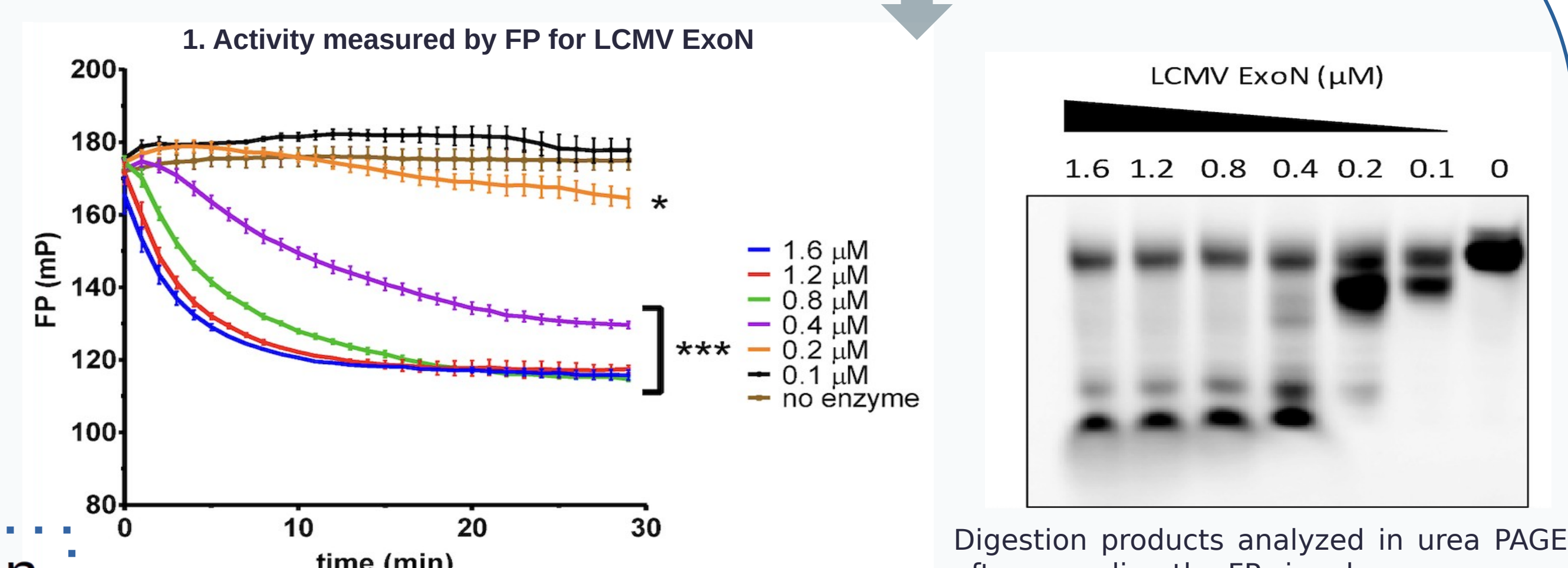
NUCLEASES INVOLVEMENT IN VIRAL LIFE CYCLE

Endonuclease of L involved in cap-snatching mechanism (transcription)

Exonuclease involved in controlling innate immunity

→ 2 enzymes 1 mode of action

Arenavirus pipe-line



CONCLUSIONS & PERSPECTIVES

Global comprehension of biological system is critical to achieve proper understanding of the MOA of an enzyme and to develop inhibition strategy. Over the last decade we have characterized several structures and activities of the key proteins of Arenaviral replication complex, both apo and in complex with inhibitors targeting the two nucleases. Compounds targeting both viral transcription and viral impairment of innate immunity present lesser the chances of selecting resistant mutations. We proved thru transversal approach studies that fluorescence polarization (FP) method is reliable, sensitive to monitor nuclease activity and feasible in 384-well plates. The method is robust, rapid and non-destructive allowing the visualization of the RNA substrate degradation using gels as orthogonal method of confirmation. We identified several hit inhibitors of viral arenavirus (LCMV) ExoN activity and determined by fluorescence polarization their IC₅₀ at a micromolar level. Then we validated their activities in cell culture assays and we found compounds displaying efficiency at micromolar level against Junin virus a new world Arenavirus. These results highlight the effectiveness of translational approach in the screening of compounds libraries for the discovery of antivirals. Structural characterization of the compound in complex with the ExoN is underway.

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