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

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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 ribonucleoproteine 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 caries 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 theses activities[2]. We present a global study aiming to characterize the assembly of the NP[3], through flexible domains[4], a step critical for vRNApackaging and the polsitioning 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[67]. 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].

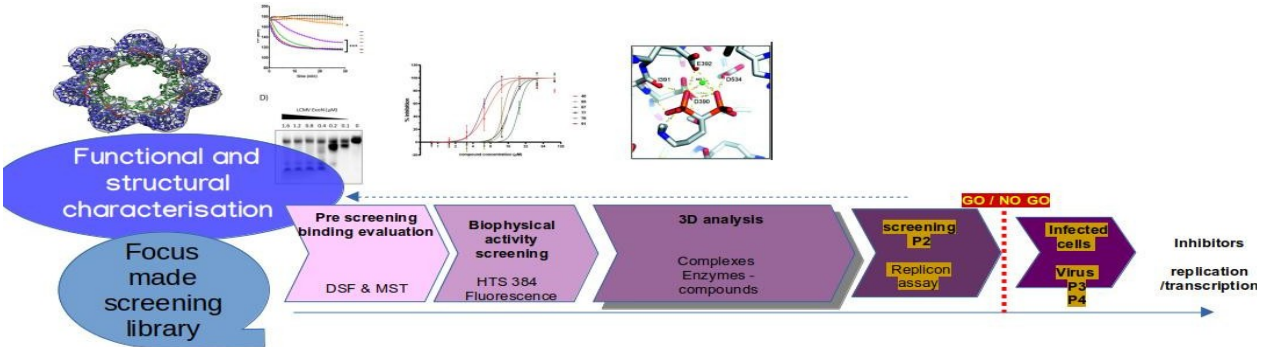

Figure 1. Experimental pipeline from characterisation to inhibitor.

[1] Ferron F, Weber F, de la Torre JC, Reguera J. Virus Res. 2017 Apr 15;234

[2] Yekwa E, Khourieh J, Canard B, Papageorgiou N, Ferron F. Acta Crystallogr D Struct Biol. 2017 Aug 1;73(Pt 8):641-649

[3] Papageorgiou N, Vaitsopoulou A, Diop A, Nguyen THV, Canard B, Alvarez K, Ferron F. FEBS Open Bio. 2021 Apr;11(4):1076-1083.

[4] Papageorgiou N, Spiliopoulou M, Nguyen TV, Vaitsopoulou A, Laban EY, Alvarez K, Margiolaki I, Canard B, Ferron F. Viruses. 2020 Jul 17;12(7):772

[5] Hernández S, Feracci M, De Jesus CT, El Kazzi P, Kaci R, Garlatti L, Mondielli C, Bailly F, Cotelle P, Touret F, de Lamballerie X, Coutard B, Decroly E, Canard B, Ferron F, Alvarez K. Antiviral Res. 2022 Aug;204:105364.

[6] Saez-Ayala M, Yekwa EL, Carcelli M, Canard B, Alvarez K, Ferron F. IUCrJ. 2018 Feb 22;5(Pt 2):223-235.

[7] Nguyen THV, Yekwa E, Selisko B, Canard B, Alvarez K, Ferron F. IUCrJ. 2022 May 28;9(Pt 4):468-479

[8] Saez-Ayala M, Laban Yekwa E, Mondielli C, Roux L, Hernández S, Bailly F, Cotelle P, Rogolino D, Canard B, Ferron F, Alvarez K. Antiviral Res. 2019 Feb;162:79-89.