Universitat Autònoma de Barcelona
Bellaterra, Barcelona, Spain
4rt grau de bioquímica
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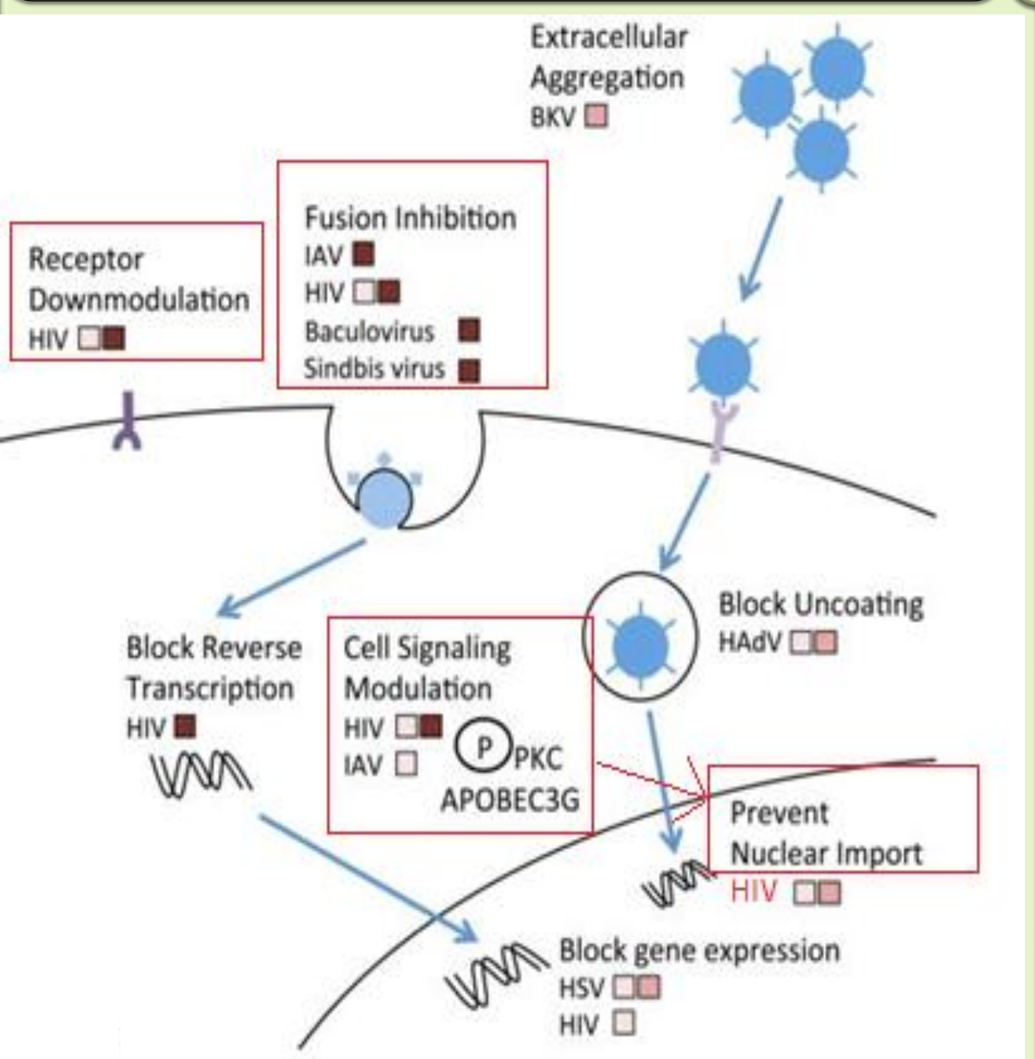
Alumne:Albert Díaz Jimeno

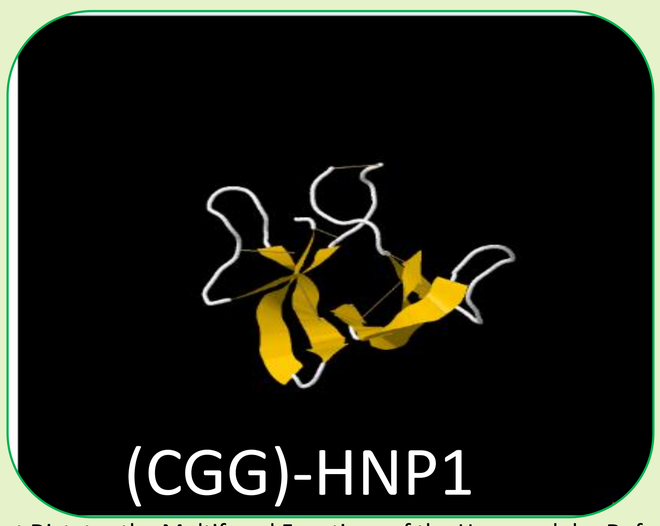
Tutor: Mohammed Moussaoui

Does covalently modified HNP1 have a better anti-HIV activity than HNP1wt?

INTRODUCTION: HNP1wt anti-HIV mechanisms



Modified from : **Antiviral Mechanisms of Human Defensins** Sarah S. Wilson,* Mayim E. Wiens,* and Jason G. Smith# J Mol Biol. 2013 Dec 13; 425(24): 10.1016/j.jmb.2013.09.038.



What Dictates the Multifaced Functions of the Human alpha-Defensin HNP1?Wei, G., de Leeuw, E., Pazgier, M., Rajabi, M., Li, J., Zou, G., Ericksen, B., Wu, Z., Yuan, W., Szmacinski, H., Lu, W-.Y., Lubkowski, J., Lehrer, R.L., Lu, W. Not published

Through the looking glass, mechanistic insights from enantiomeric human defensins.Wei, G., de Leeuw, E., Pazgier, M., Yuan, W., Zou, G., Wang, J., Ericksen, B., Lu, W.Y., Lehrer, R.I., Lu, W.
Journal: (2009) J.Biol.Chem. **284:** 29180-29192

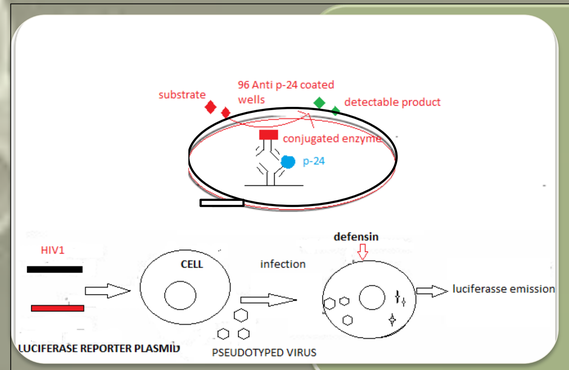
BUDGET (NOT TAKING IN ACCOUNT PERSONAL COSTS):

- 40855,2€

DURATION OF THE PROJECT:

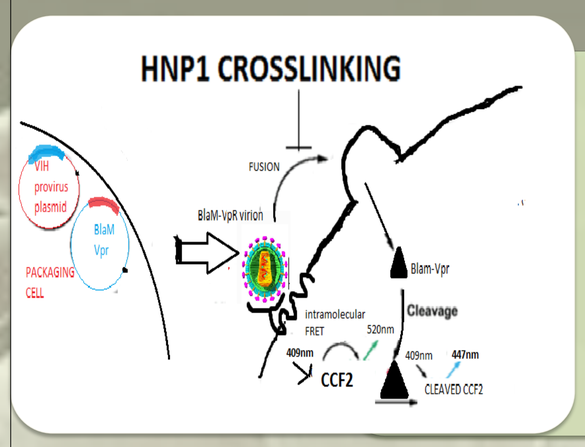
- 24 MONTHS

OBJECTIVE: Evaluation of (CGG)-HPN1 anti-HIV activity



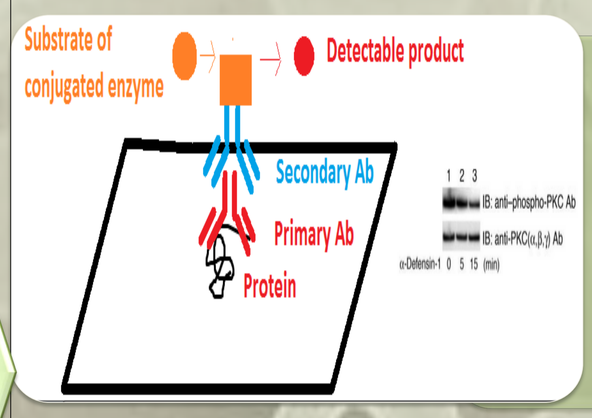
INFECTIVITY ASSAY

- p24 ELISA: the defensin's effectivity will be compared by taking de supernatant of the infected cells at 48 hour post infection and comparing the p24 levels of antigen through ELISA.
- LUCIFERASE REPORTER: pseudotyped HXB2 and JFRL replication defective viruses with luciferase reporter will be use in order to asses the antiviral capacity of the defensins



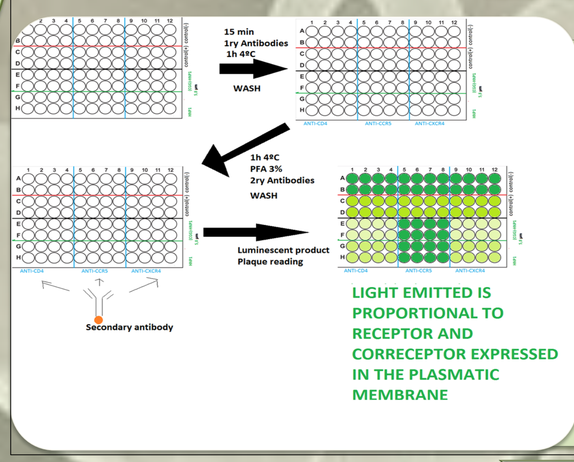
FUSION INHIBITION ASSAY

- HNP1 oligomerizes at the membrane crosslinking the HIV and its receptors to other membrane proteins preventing the fusion. Here we'll be using the BlaM assay to assess whether or not the modified defensin is more effective than the wild type in this particular inhibition mechanism.



PKC INHIBITION ASSAY

- One of the inhibition mechanisms of HNP1 wt consist in PKC inhibition by impeding its phosphorilation
- To observe the levels of PKC inhibition we'll use a western blot against phospho-PKC(αβγ) which will show us the level of PKC activation in a HIV infection with or without defensins



CD4 ,CXCR4 AND CD5 DOWNREGULATION

- The expression of the receptor and correceptors of HIV will be measured by immunofluorescence staining, using specific receptor and correceptor downregulators to compare their activities with HNP1 and (CGG-HNP1)

INFECTIVITY ASSAY:

The original HNP1 could inhibit most HIV strains around a IC50 1,2µM so if the covalently tethered defensine is really more efective should she an IC50< <1,2µM in both the p24 and the luciferase reporter aproaches

FUSION INHIBITION:

The Blam assay uses the luminescence ratio(520nm/477nm) of two FRET emitting compunds to determine whether the HIV has entered in the cell or not. Therefore we should see in the sample treated with CGG-HNP1 a larger luminescence ratio at the same concentration of defensin

EXPECTED OVERALL RESULT: increase in anti-HIV activity due to modification

CD4 ,CXCR4 y CD5 DOWNREGULATION:

If we were having positive results we should observe less intense immunostaining in the cells treated with (CGG)-HNP1 compared with the cells treated with HNP1wt , meaning that in the former the expression of receptors and correceptor has been more downregulated than in the latter.

PKC INHIBITION:

If the modified defensive does have a better PKC inhibition activity we should see less PKC phosphorilation having equal time of incubation and equal concentration of defensin