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Inhibiting HIV-1 reverse transcription by target the reverse transcription complex

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Retroviruses & Novel Drugs
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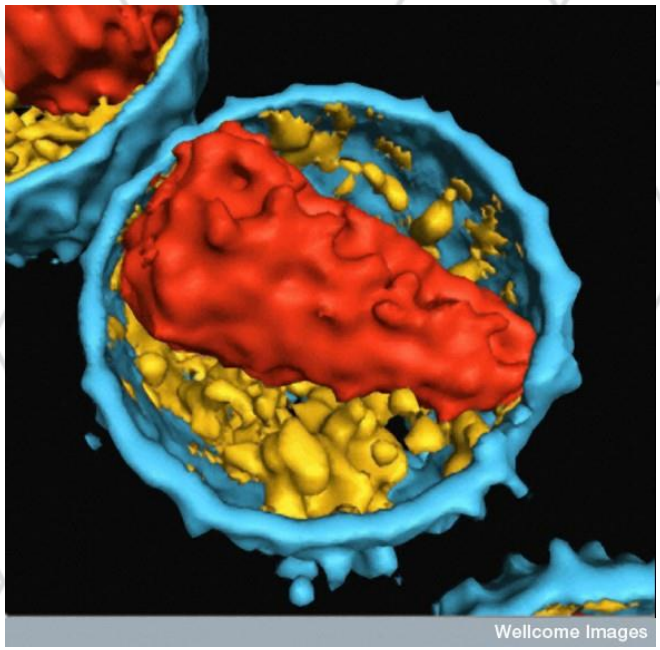
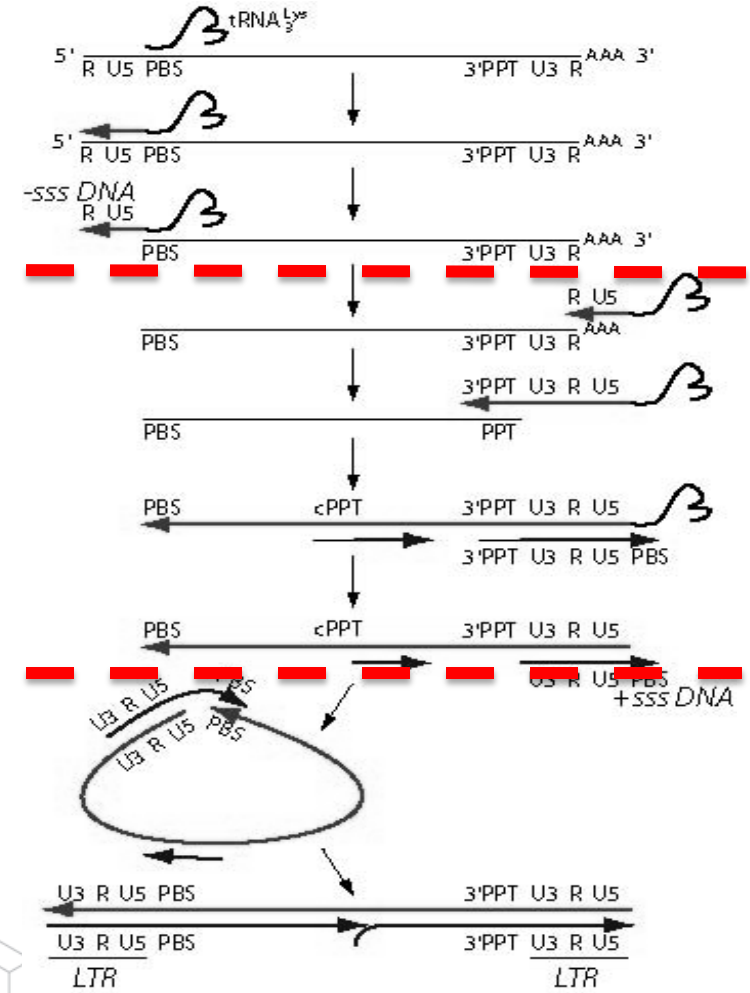


Reverse Transcription:
+single strand RNA to double strand DNA

Early:

Intermediate

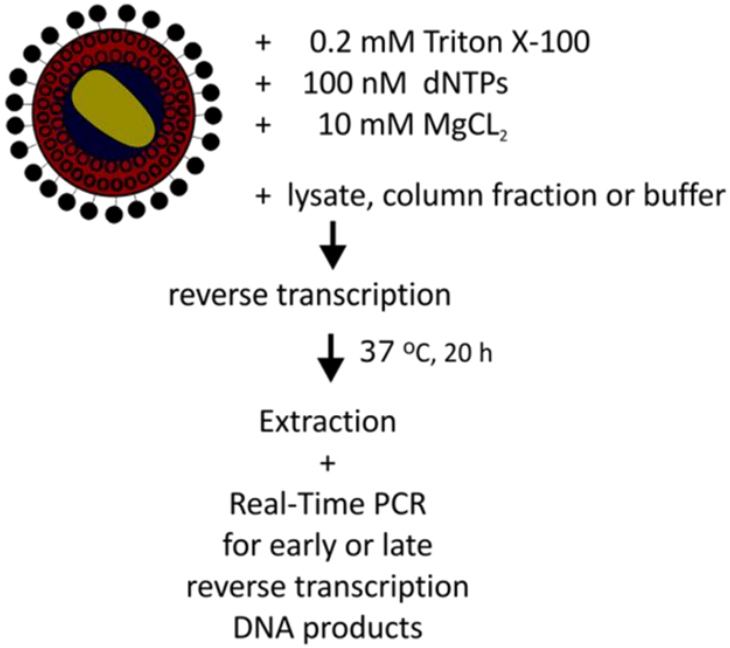
Late:



Background:
Cellular factors stimulate reverse transcription in vitro

- Endogenous Reverse Transcription (ERT) is inefficient (late DNA/early DNA products) compared to reverse transcription in cells. ***(Hooker et al. 2003)*** ***(Warrilow et al JVI 2008)***
- ERT efficiency can be stimulated by cell lysates. (***Narayan et al., PNAS 2004)*** ***(Warrilow et al., JVI 2008)***

Late DNA:Early DNA



Background 2:
Eukaryotic translation elongation factors stimulates reverse transcription in vitro

- eEF1A and other EF1 subunits were identified in cell lysate fractions that simulate reverse transcription late DNA synthesis *in vitro*.
- The immunodepletion of eEF1A and eEF1G from lysate fractions resulted in decreased efficiency of endogenous reverse transcription reactions.

Warren et al PNAS 2012

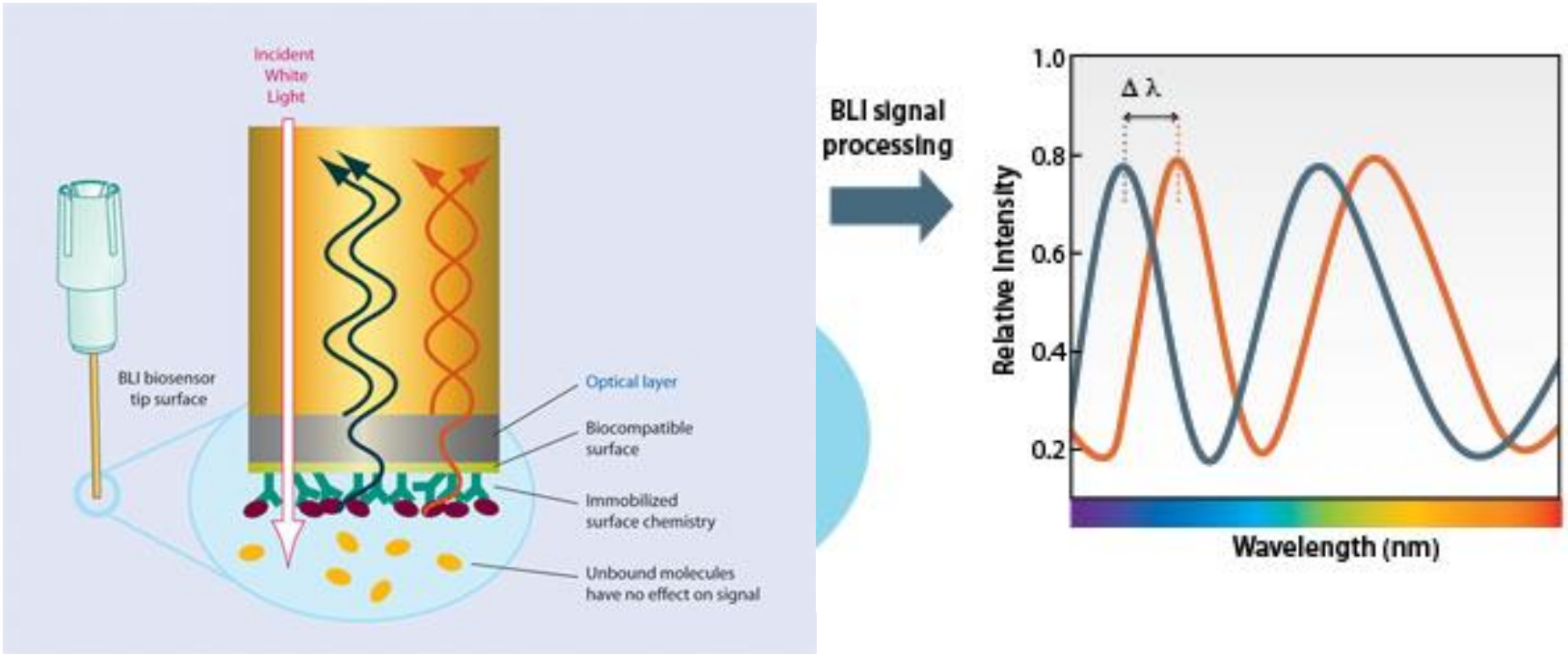
Background 3:
eEF1A and eEF1G associate with the reverse transcription complex subunits

- RT and IN associated with eEF1A or eEF1G by co-IP.
- eEF1A/1G co-purified with RTC isolated by isopycnography
- siRNA downregulated eEF1A/1G in cells decreased post-infection levels of RTC, suggesting that one or both are important for RTC stability

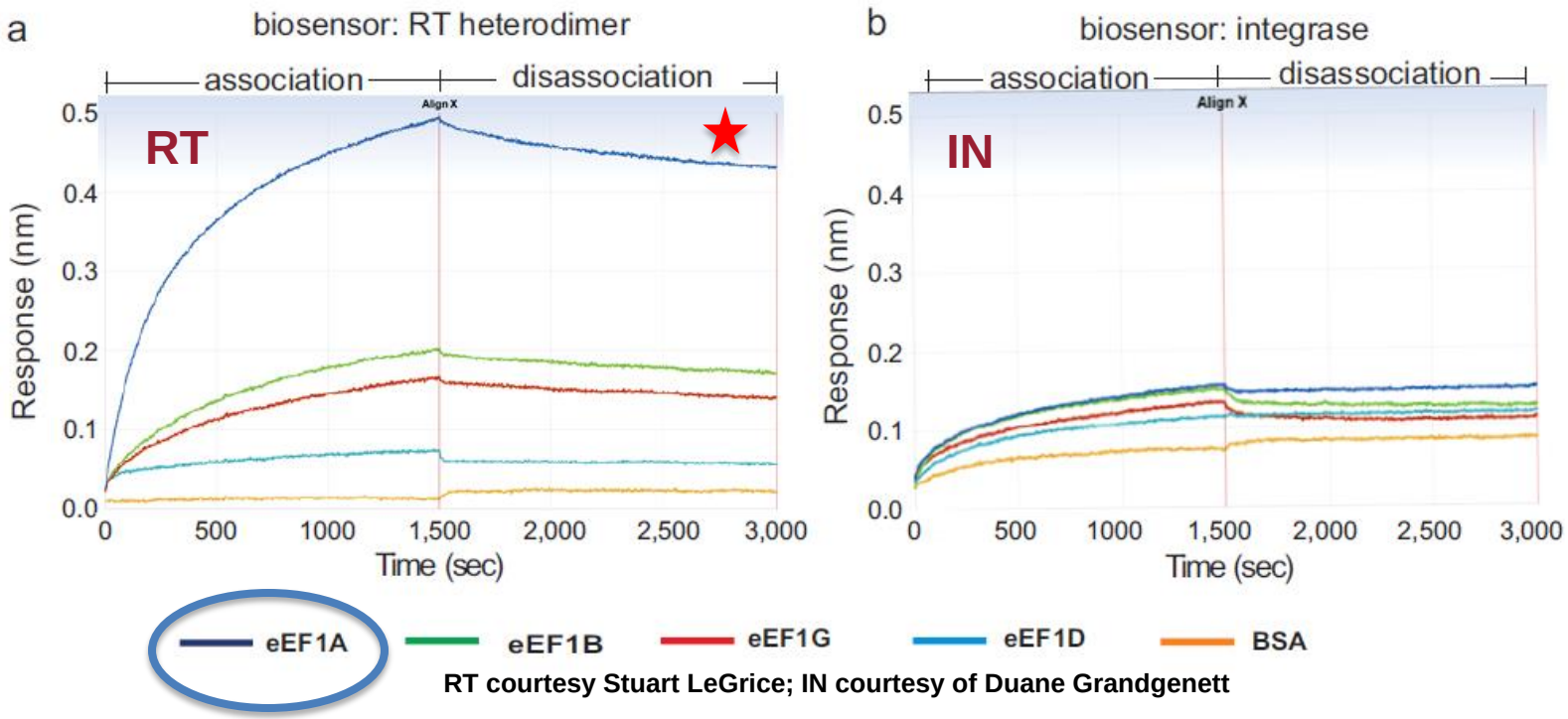
Warren et al PNAS 2012

Does eEF1A or 1G directly interact with the RTC subunits RT or IN?

Biolayer Interferometry (BLI)



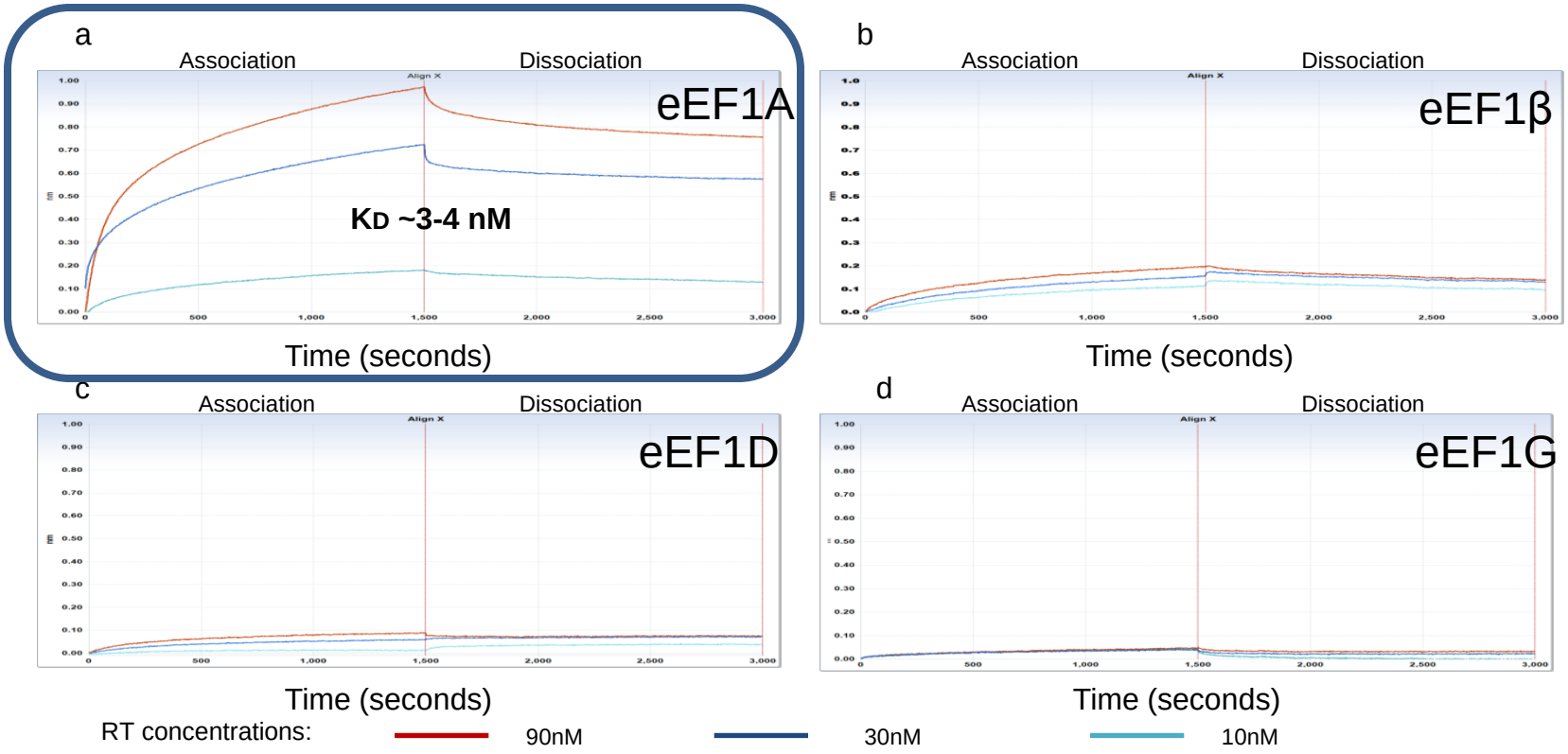
The EF1 subunit eEF1A binds RT not IN *in vitro* using BLI



RT courtesy Stuart LeGrice; IN courtesy of Duane Grandgenett

Preliminary data

Reciprocal experiments using EF1 subunit probes and soluble RT

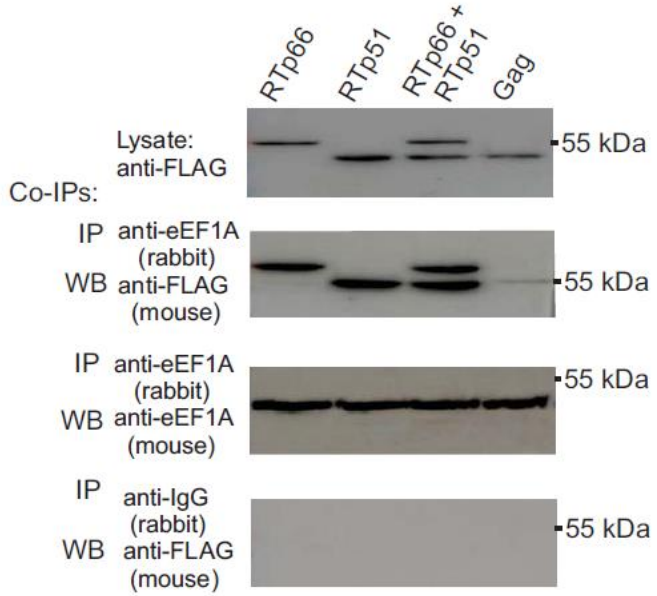


Sensorgram of association and dissociation of eEF1A1, eEF1B, eEF1D and eEF1G with HIV RT. Biotinylated (a) eEF1A1, (b) eEF1B, (c) eEF1D and (d) eEF1G were immobilized on biosensors. The association and dissociation with various concentrations of HIV RTp66/p51 were measured respectively on the OctetRed system. Data are representative of three independent experiments

Preliminary data

Co-IP assays indicate interaction between RT and eEF1A

IP: eEF1A WB: RT



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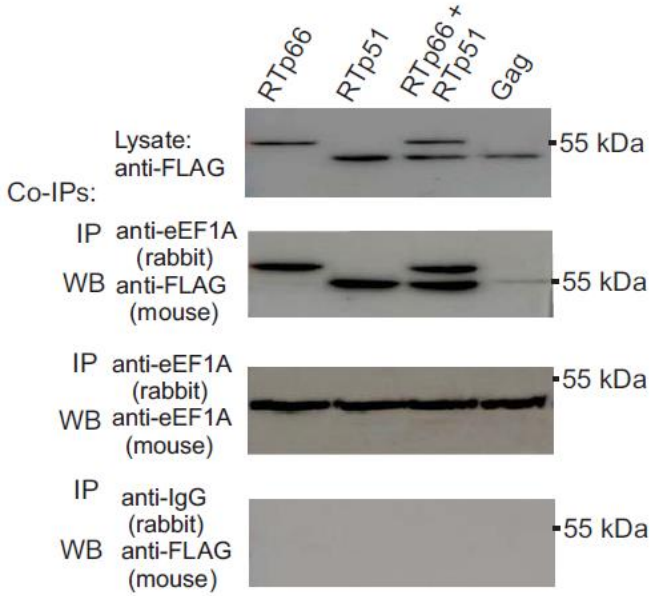
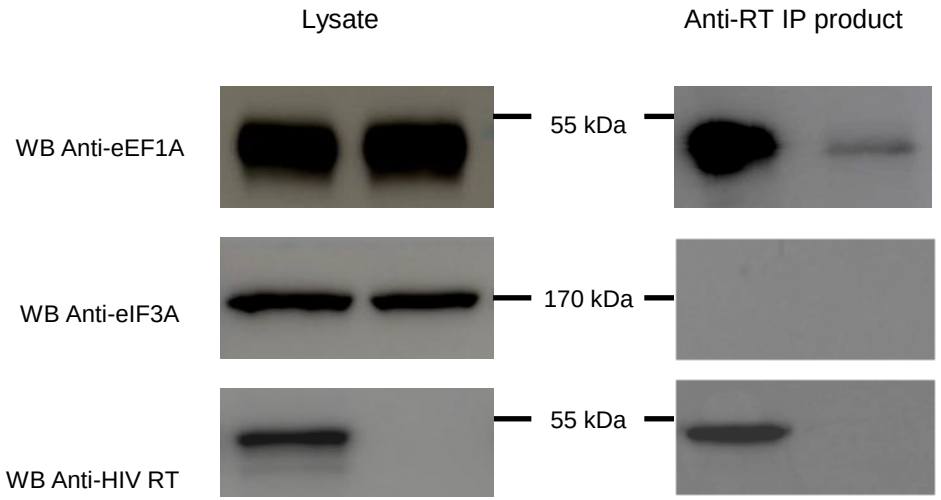
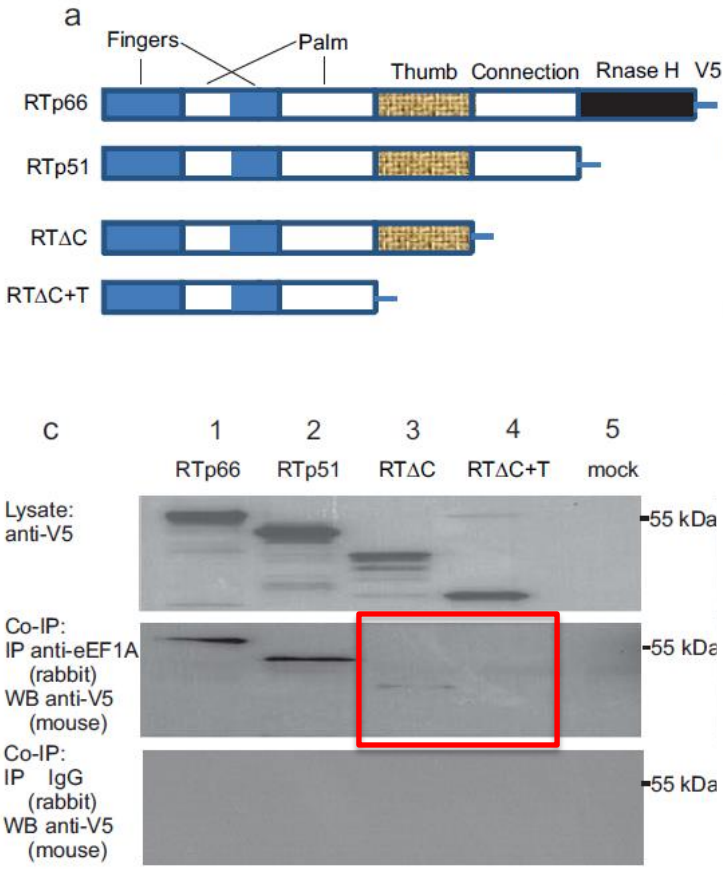


IP: RT WB:eEF1A



Preliminary data

The RT thumb and connection domain are important for interaction with eEF1A



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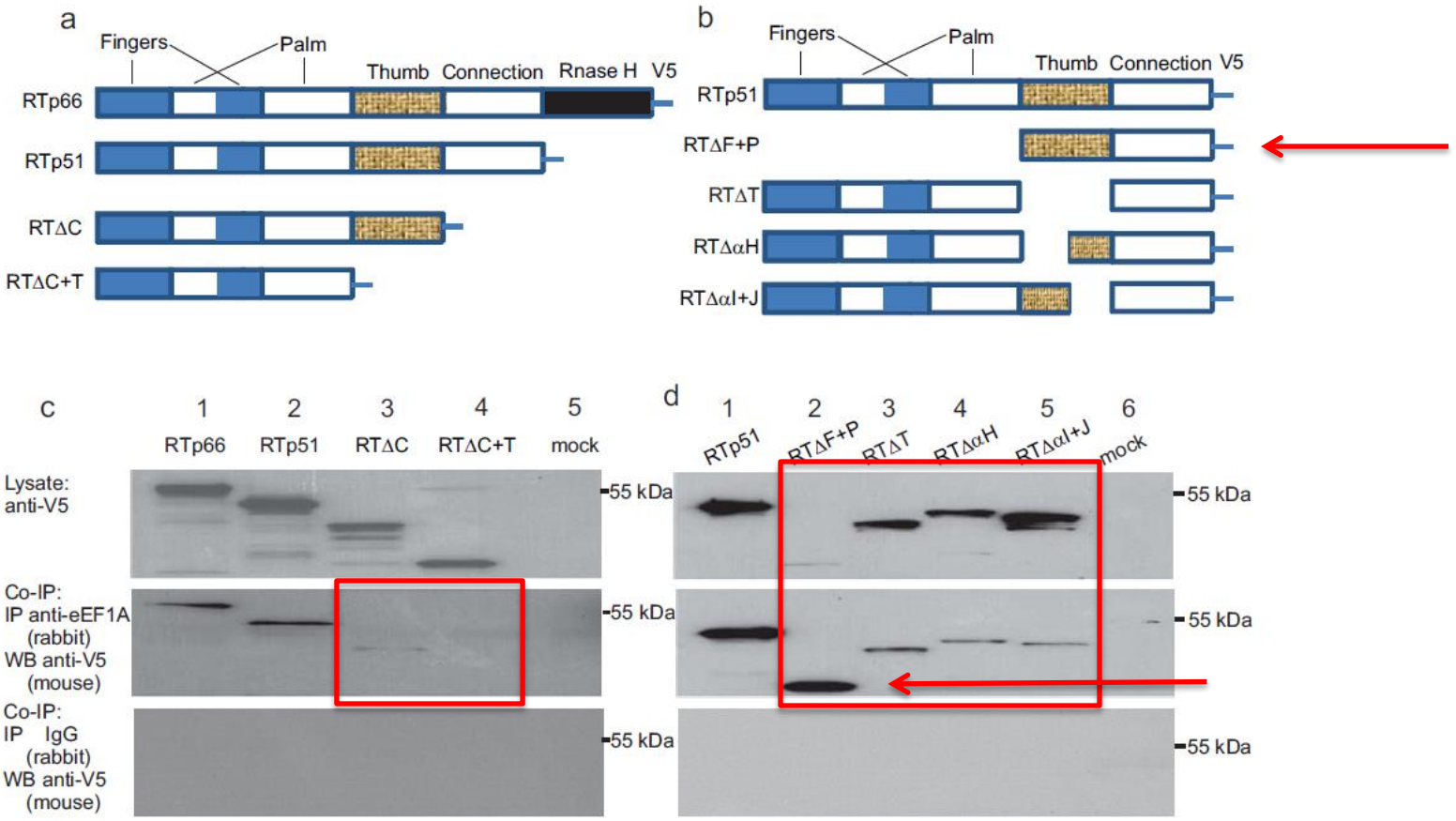
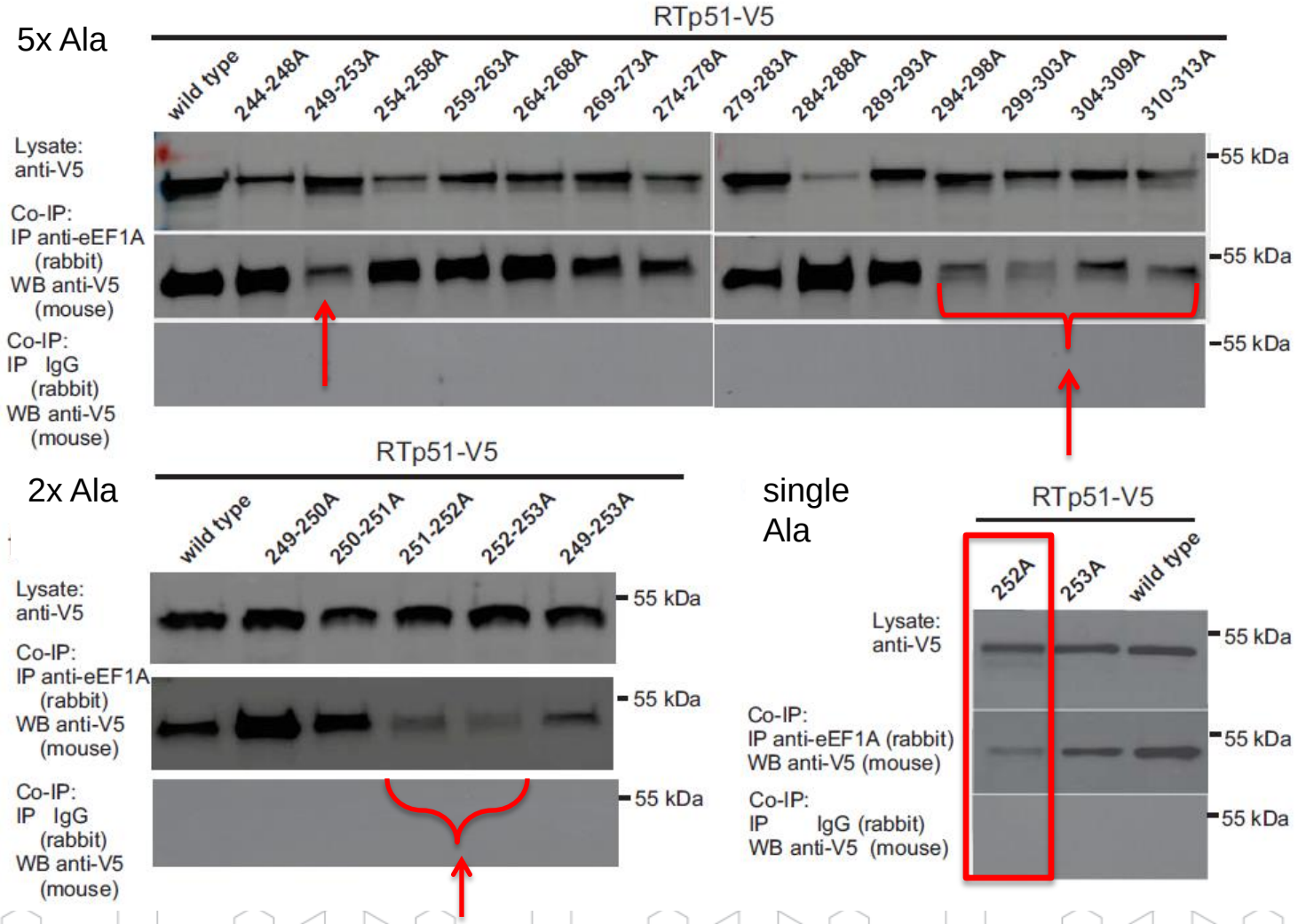


Preliminary data

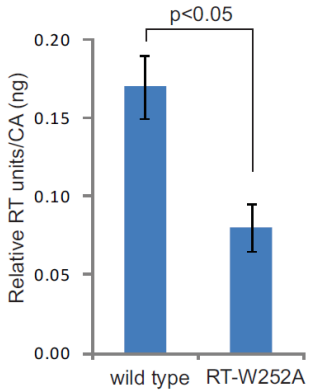
Alanine scanning mutagenesis of the RT thumb domain: multiple binding sites?



Preliminary data

Does W252A affect RT activity / reverse transcription?

Standard homopolymer substrate RT assay.



Reverse Transcription in Jurkat cells; 4 hours post-infection

Table 2: Analysis of reverse transcription by wild type or RT-W252A mutant virus in Jurkat cells

		WT[1,3]	W252A[2,3]	p value[4]	WT + NVP[5,3]
Infection using equivalent amount of CA[7]	early DNA copy number	15,212 ± 3,226	11,350 ± 3,110	≥ 0.05	8,393 ±3,785
	late DNA copy number	3,244 ± 696	1,332 ± 216	< 0.05	1,912 ± 598
	late/early DNA %	21.3 ± 0.2	11.7 ± 0.6		22.7 ± .06

The decrease in RTn efficiency is consistent with previous studies that indicated eEF1A is important for late DNA synthesis. **Warren et al. 2012 PNAS**

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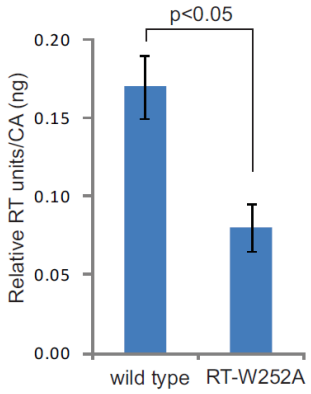

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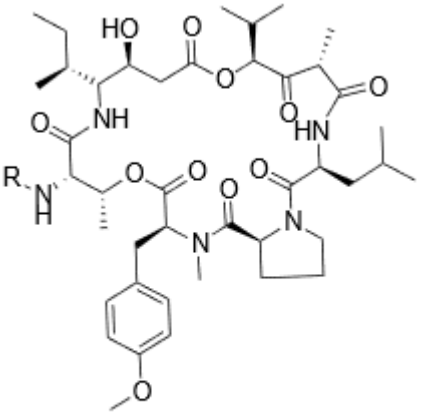
Didemnin B binds to eEF1A and inhibits its role in translation

Didemnins are cyclic depsipeptide compounds isolated from a tunicate (sea-squirt).

Didemnin B is the one that possesses the most potent biological activities.

It is a strong antiviral agent against both DNA and RNA viruses such as herpes simplex virus type 1.

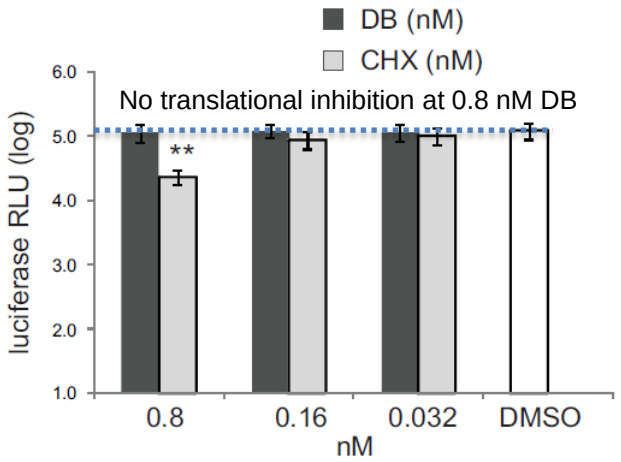
Dideminin B irreversibly binds eEF1A and therefore inhibits translation blocking interaction with the eF1B complex.

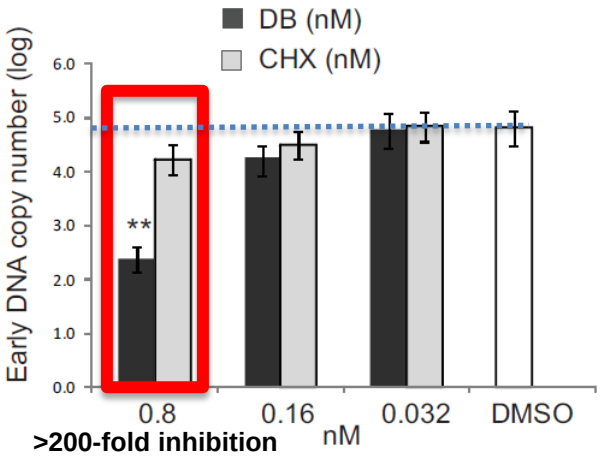


R = *N*-Me-L-Leu : Didemnin A
R = Lac-Pro-*N*-Me-L-Leu : Didemnin B
R = Lac-*N*-Me-L-Leu : Didemnin C

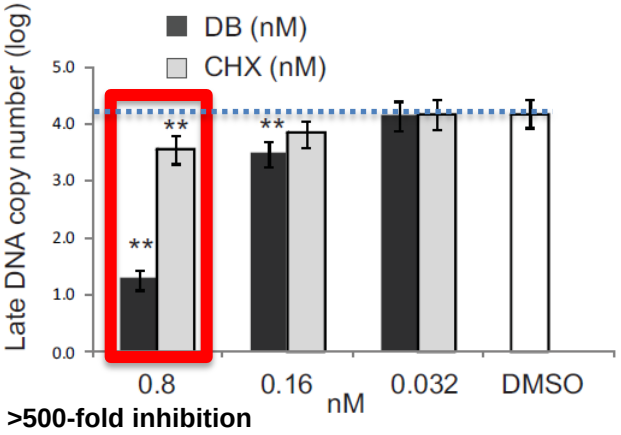
The eEF1A inhibitor, didemnin B (DB) inhibits HIV-1 reverse transcription.

1. HEK293T cells treated with DB or CHX for 2 h
2. Attach HIV-1 at 20°C for 2h
3. Infection at 37°C 4 h
4. Collect cell lysate for analysis of HIV-1 DNA

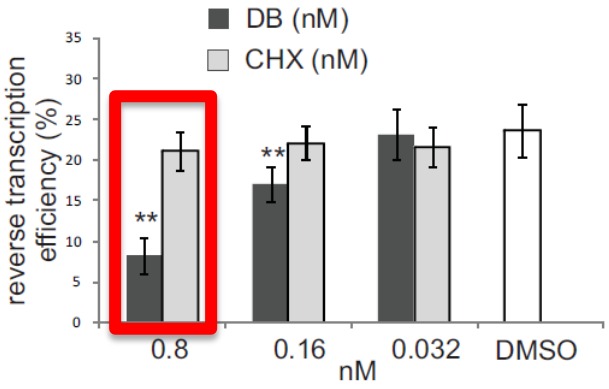




>200-fold inhibition



>500-fold inhibition

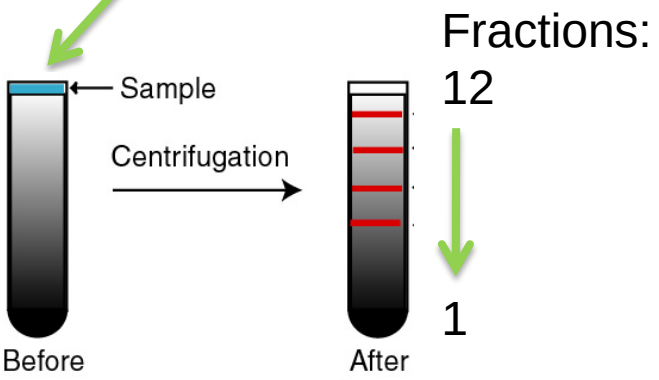


Preliminary data

Do eEF1A1 and eEF1G associate with the reverse transcription complex in cells?

- **+/- didemnin B**
- **Infected with HIV-1 pseudotyped with V-VSG envelope for 4 h. Heat-inactivated virus were used as a negative control.**

- **Cells are lysed using a dounce homogenizer**

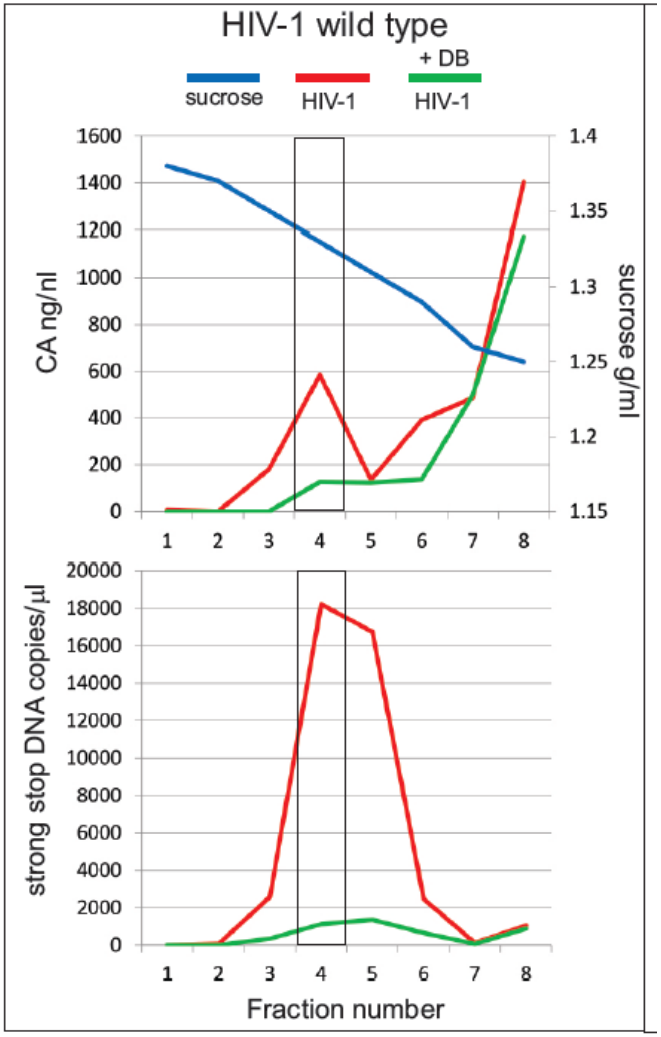


Equilibrium density gradient centrifugation using a 20 – 70 % continuous sucrose gradient

Fractions are assayed for:

1) **HIV-1 DNA by qPCR**
2) **ELISA for CA**

Analysis of RTCs +/- DB by isopycnography; 20-70% sucrose gradients



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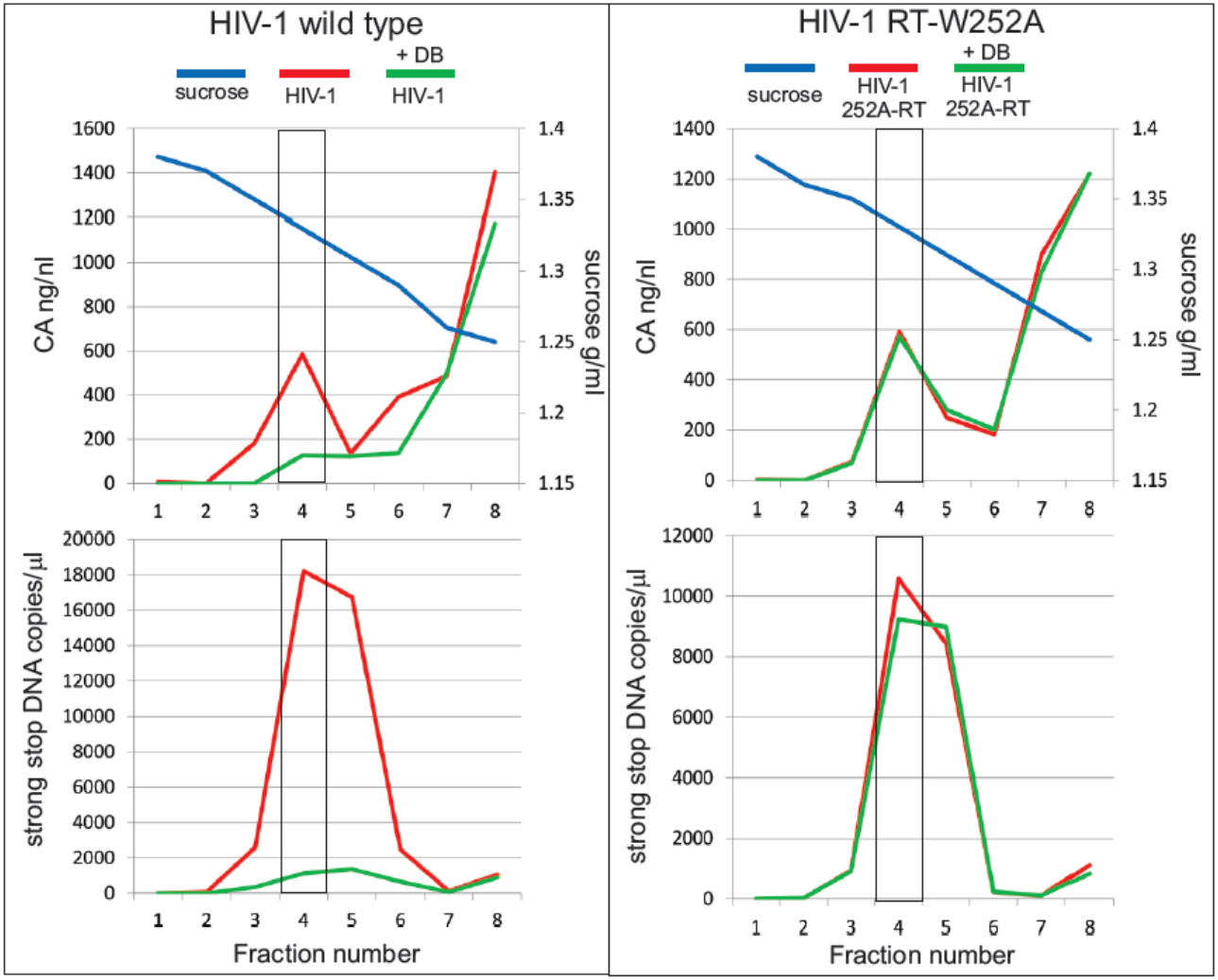
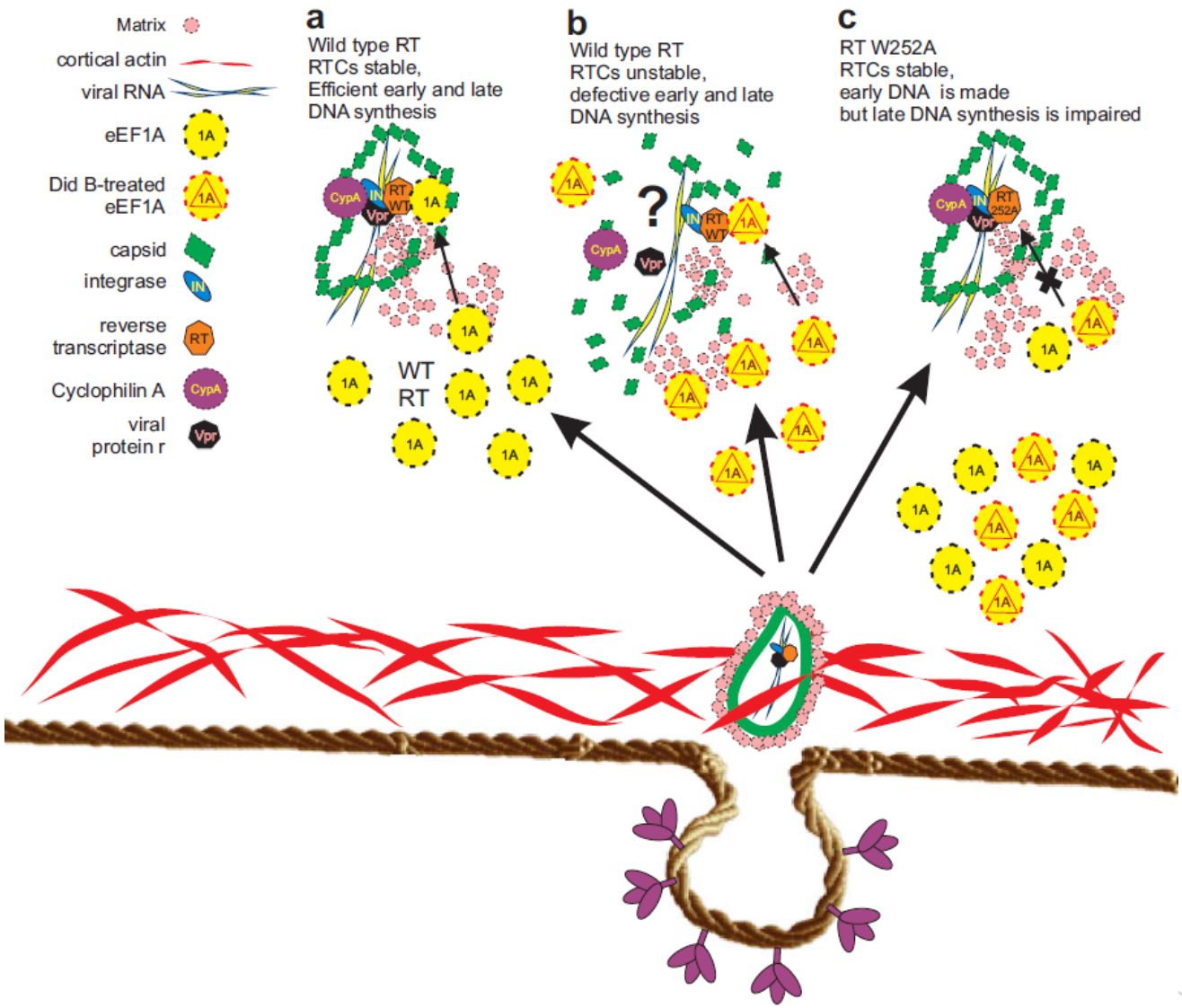
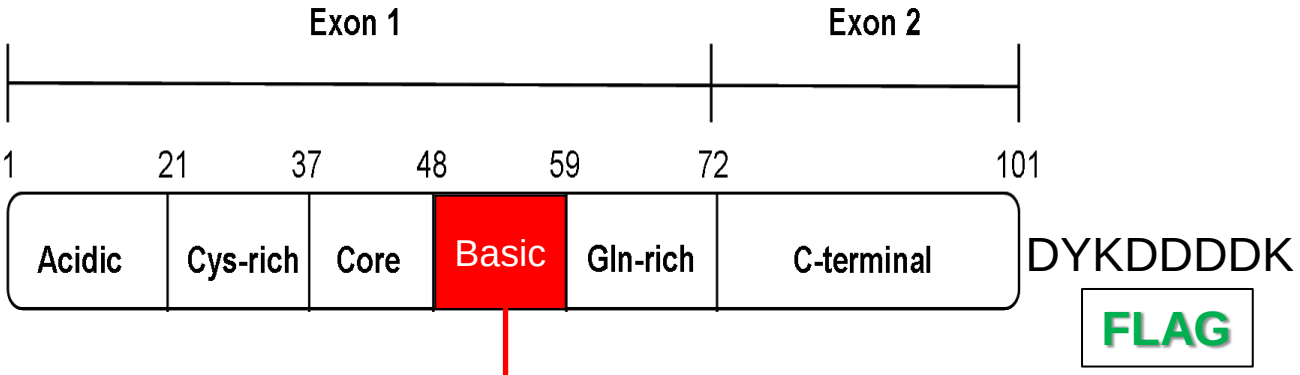


Preliminary data

Proposed model: DB-treated eEF1A leads to altered uncoating/RTN



A Tat with a mutated basic domain inhibits reverse transcription



- Basic domain which binds to the TAR sequence is mutated.

RKKRRQRRR ➡ GGGGGAGGG

- “Nullbasic”

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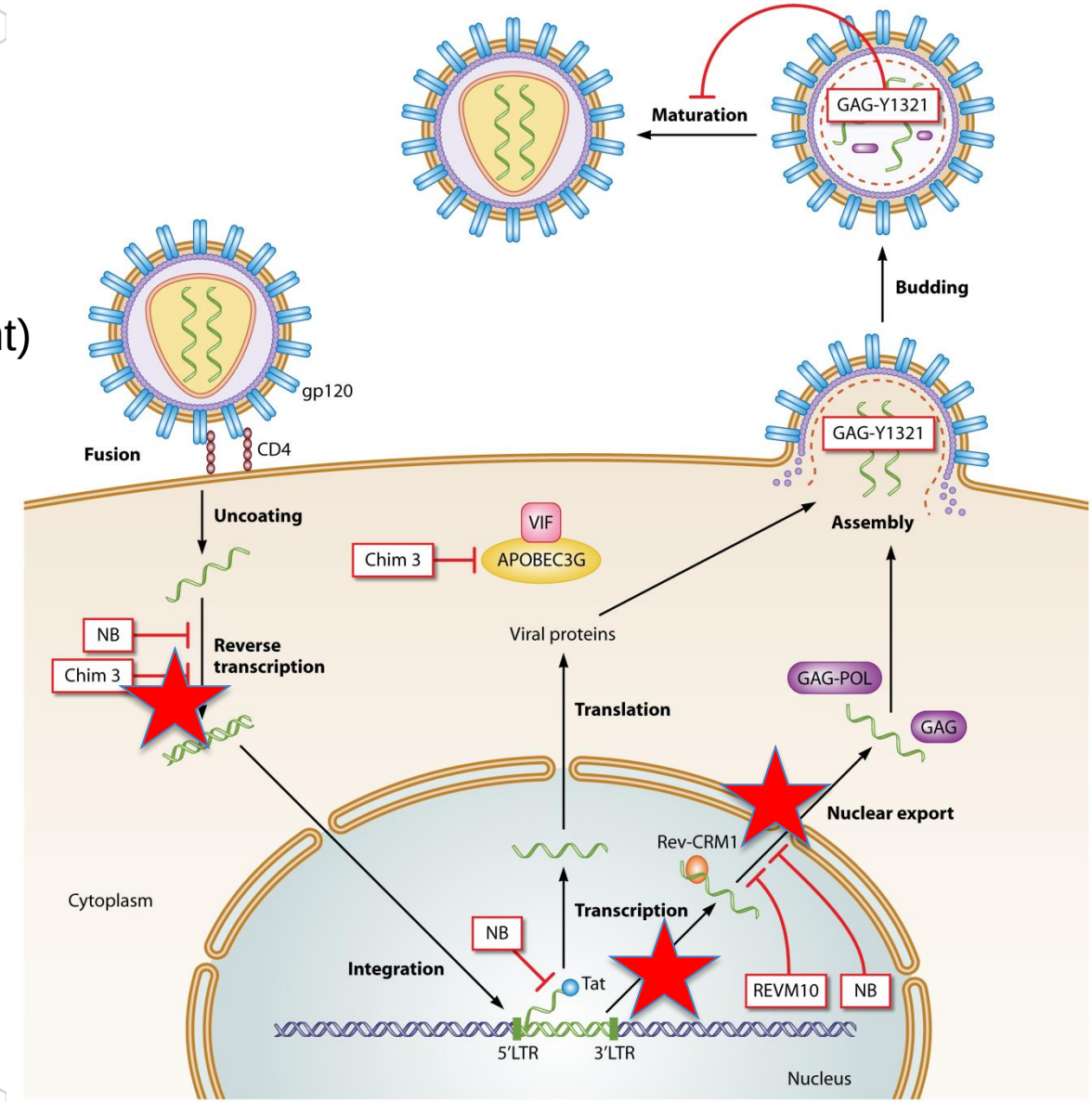
Potential Inhibition of HIV-1 Replication by a Tat Mutant

Luke W. Meredith[1,4¤], Haran Sivakumaran[1,3], Lee Major[2], Andreas Suhrbier[2,4], David Harrich[1,4]*

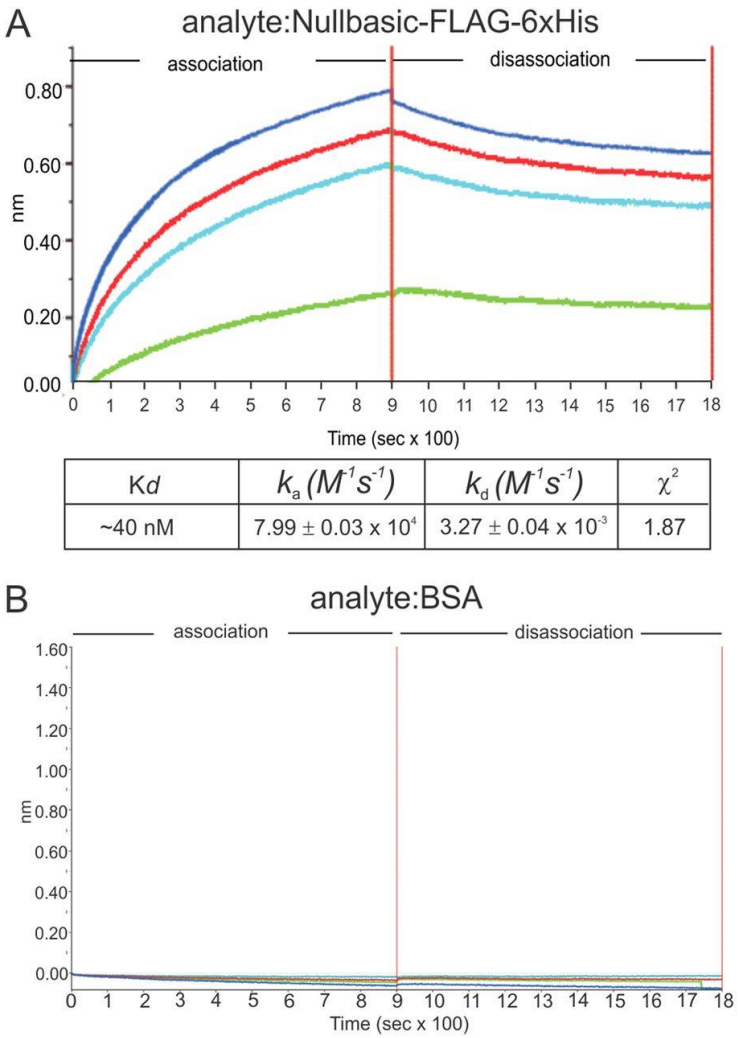
1 Division of Infectious Diseases, Queensland Institute of Medical Research, Herston, Queensland, Australia, 2 Division of Immunology, Queensland Institute of Medical Research, Herston, Queensland, Australia, 3 School of Population Health, The University of Queensland, Herston, Queensland, Australia, 4 Griffith Medical Research College, Brisbane, Queensland, Australia

Future Virology 8:757 2013

NB= Nullbasic (Tat mutant)
Inhibits RT, Tat and Rev



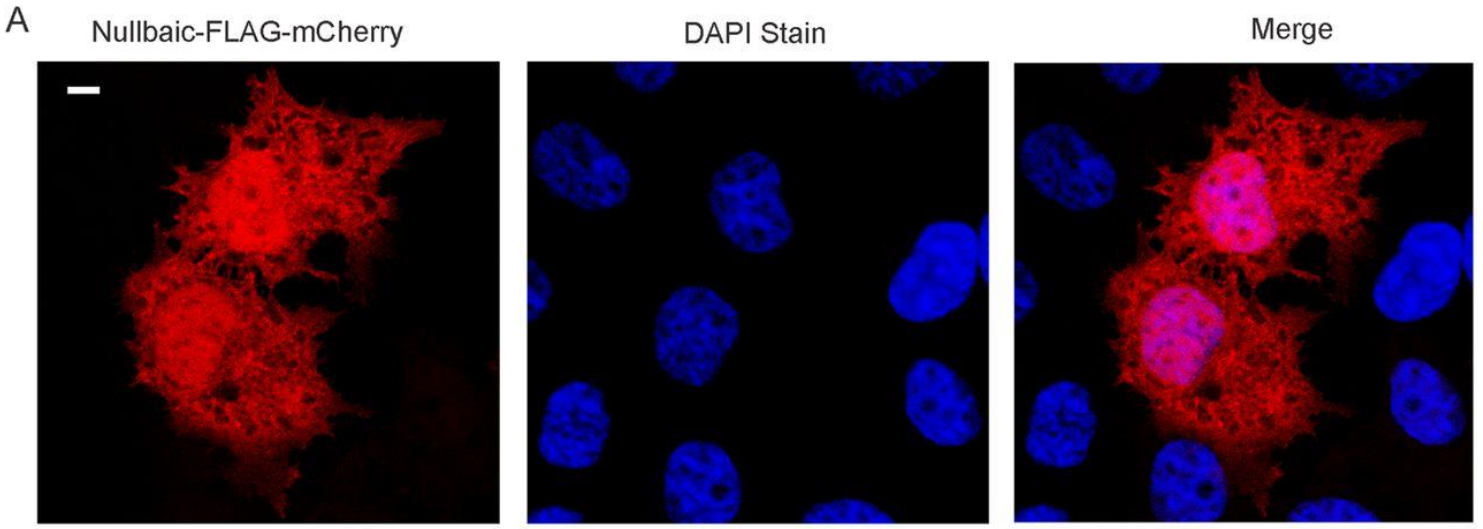
BLI sensograms of binding events between Nullbasic and RT



Kd	$k_a (M^{-1}s^{-1})$	$k_d (M^{-1}s^{-1})$	χ^2
~40 nM	$7.99 \pm 0.03 \times 10^{4}$	$3.27 \pm 0.04 \times 10^{-3}$	1.87

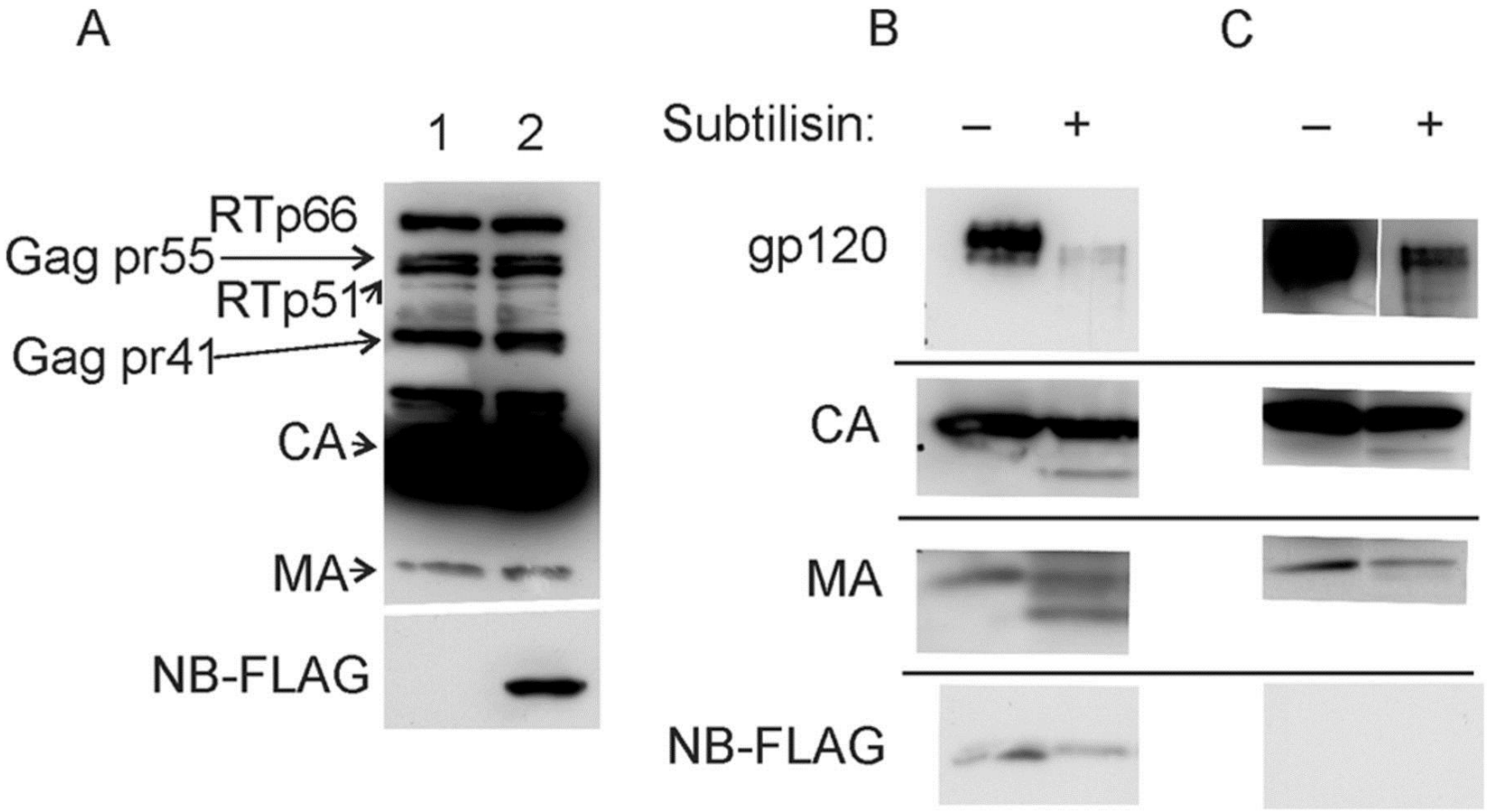
Min-Hsuan Lin et al. J. Virol. 2015;89:4827-4836

Nullbasic is located throughout the cell



Min-Hsuan Lin et al. J. Virol. 2015;89:4827-4836

Subtilisin digestion of HIV-1 indicates Nullbasic is packaged in virions



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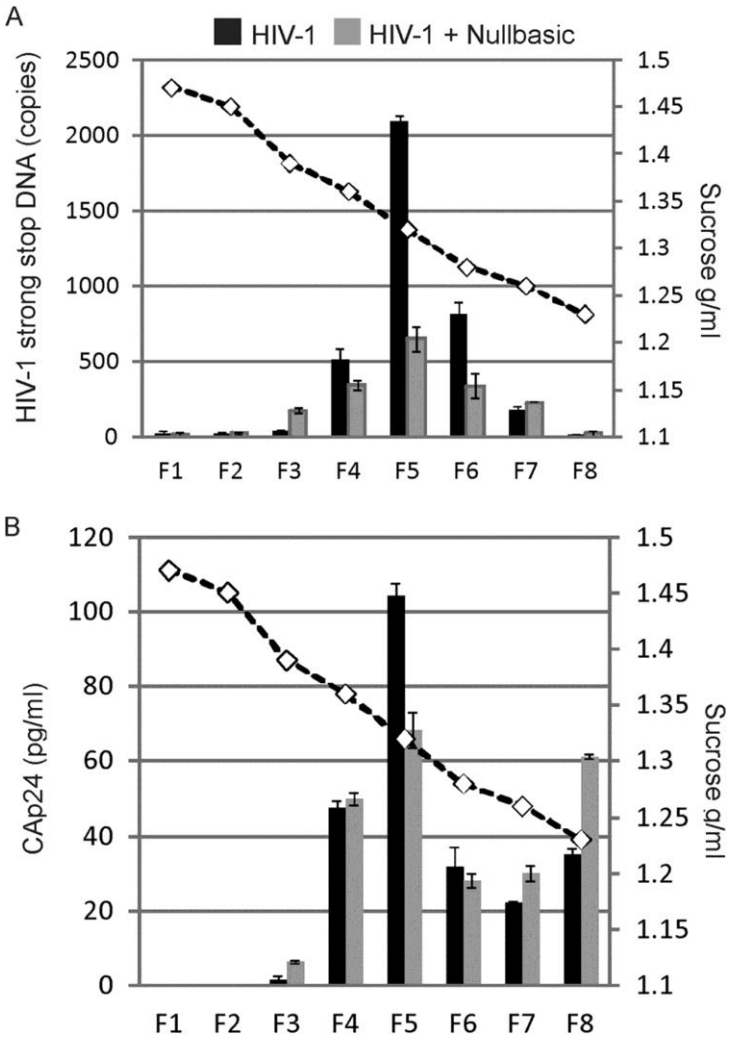
Inhibition of RTC DNA synthesis by Nullbasic



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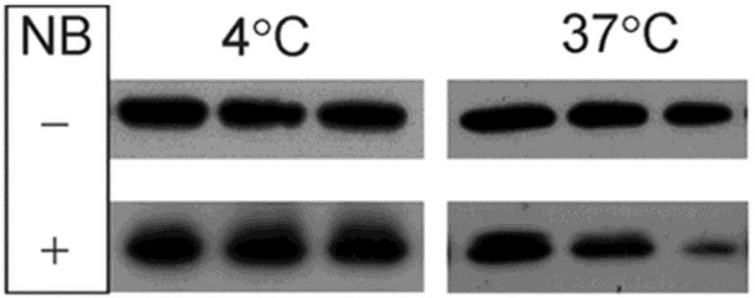
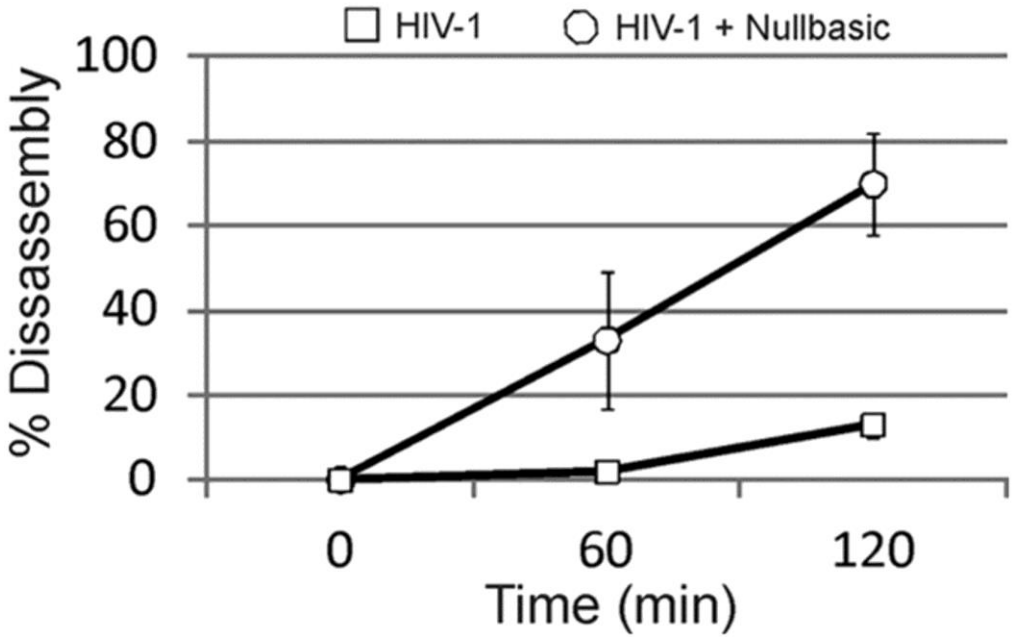
Journal of Virology

Nullbasic incorporation in HIV-1 leads to increased core disassembly kinetics in vitro.

1. Isolate intact cores from virions
2. Incubate cores at 4°C or 37°C
3. Centrifuge sample
4. Measure pelleted and soluble CA





Min-Hsuan Lin et al. J. Virol. 2015;89:4827-4836

Summary

•As eEF1A is abundant and binds RT strongly,

we propose that it is a predominant cellular RT binding protein.

•The RT thumb and connection domain contain the eEF1A binding site(s).

•Didemnin B (DB) is an eEF1A binding compound that potently inhibits reverse transcription.

•DB treatment greatly affected the levels of RTC in infected cells. However W252A RTC are not affected.

•This suggests that DB binding RT leads inappropriate uncoating and decreased reverse transcription.

•A mutant Tat protein, Nullbasic, binds to RT in virions causing instability of the viral core and defective reverse transcription.

Conclusion:

•The RTC can be destabilized by 1) small molecules that bind eEF1A1 and 2) by Nullbasic that binds RT, both events resulting in defective reverse transcription.

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Min-Hsuan Lin

Thank You !

Sir Albert Sakzewski Virus Research Centre
Celebrating 25 years of Research: 1987-2012

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Johnson Mak

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AID Australian Infectious Diseases Research Centre