Jean Lab - Current Projects

CURRENT PROJECTS

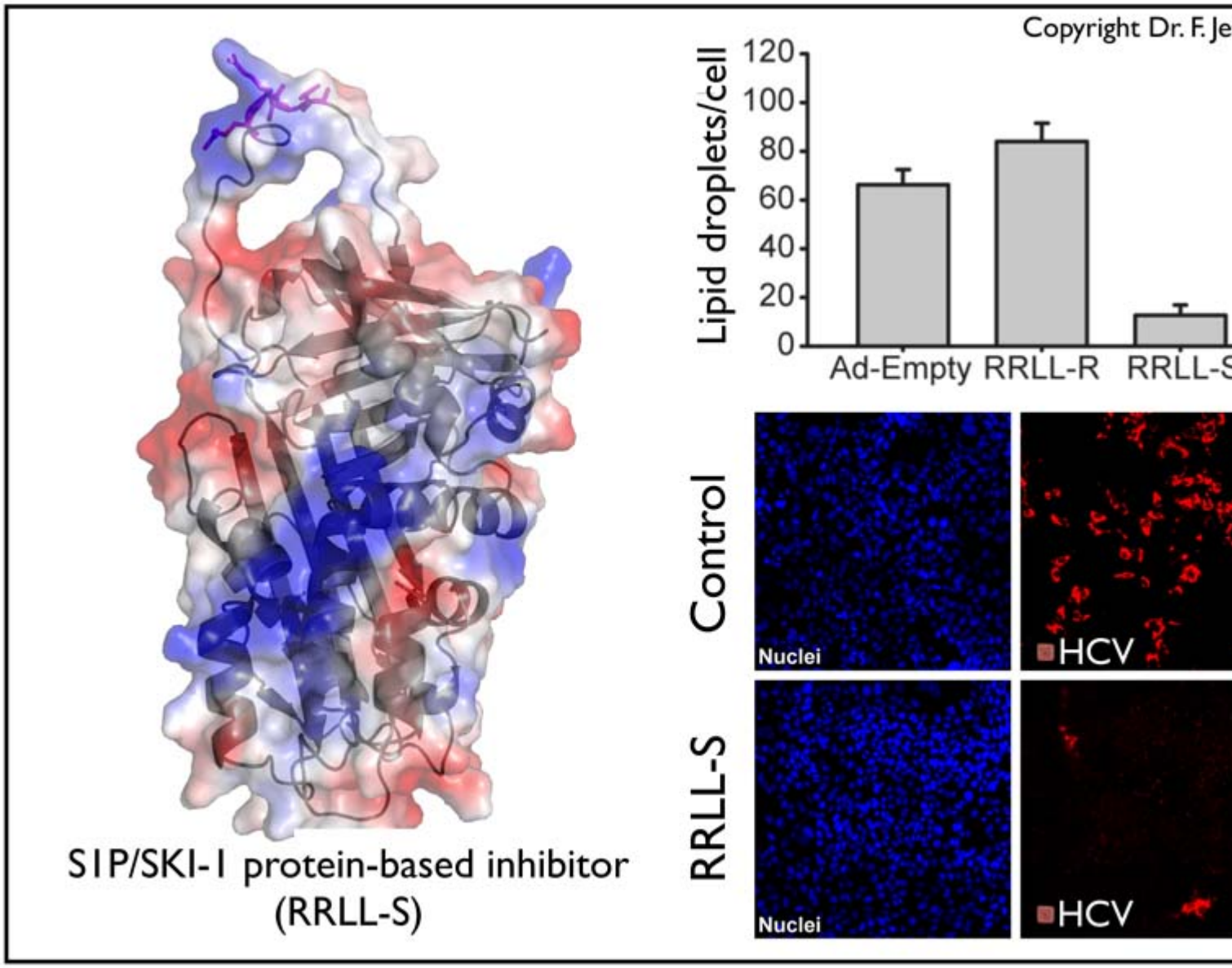
· ***Hijacking of host cell pathways by human enveloped viruses and discovery of novel indirect-acting antiviral agents (IAAAs)***

· ***Discovering novel direct-acting antiviral agents (DAAs) targeting virally encoded proteases***

· ***Exosomal microRNAs as master regulators of viral infection and blood-based diagnostic biomarkers of human viral diseases***

· ***Next generation molecular diagnostics for emerging viral diseases***

Hijacking of Host Cell Pathways by Human Enveloped Viruses and Discovery of Novel Indirect-Acting Antiviral Agents (IAAAs)



Host cell site-1 protease (S1P)/SKI-1 as master regulator of hepatitis C virus (HCV) infection: From lipid droplet biogenesis to broad-spectrum antivirals. Inhibiting S1P activity with our protein-based inhibitor (RRLL-S) completely blocked HCV infection of hepatoma cells in a dose-dependent manner. Thus, targeting host lipid droplets (LDs) biogenesis by inhibiting S1P/SKI-1 may have far-reaching applications in the therapeutic treatment of many important *Flaviviridae* viruses. In the case of HCV, overstimulation of host lipid metabolism in the liver during viral infection promotes cholesterol intracellular storage in host LDs, a critical cellular event for HCV replication, assembly, and budding (see Olmstead, A. D., et al**. Jean, F.** (2012) ***PLoS Pathogens****.* 8(1): e1002468).

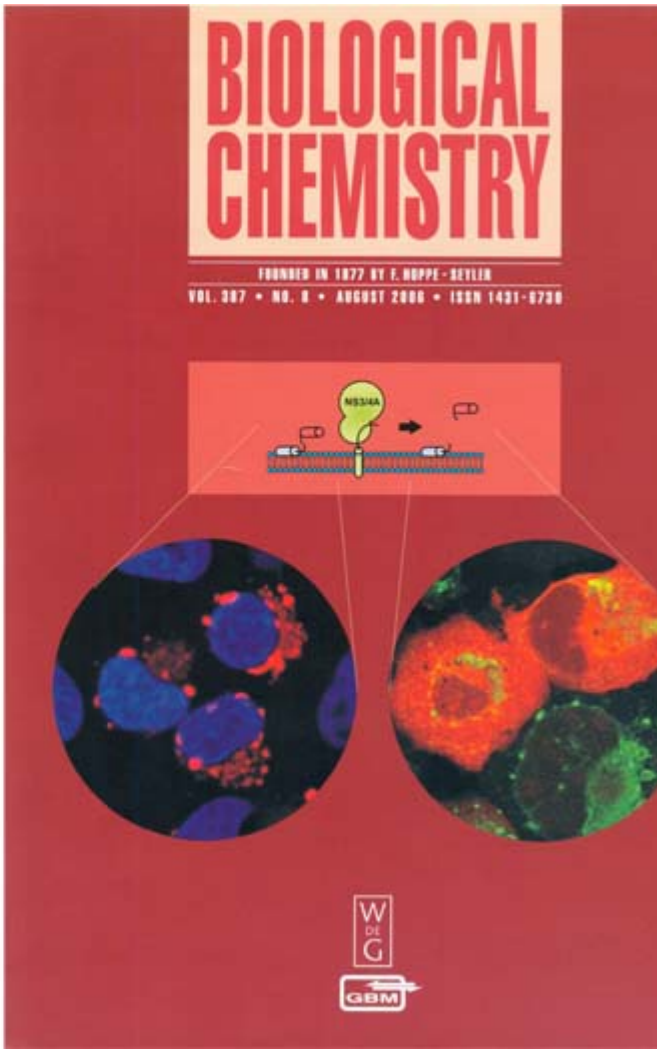
Project summary: Today?s treatment of viral diseases involves a multi-drug regimen largely based on viral enzyme inhibition and using the so-called *direct-acting antivirals* (DAAs). Although research related to the development of new DAAs is dramatically expanding and under

investigation in clinical trials, these multidrug regimens are limited by increasing multi-class drug resistance. About half of the drugs sold worldwide are small-molecule inhibitors directed at virally encoded enzymes (e.g., proteases, helicases, polymerases), and a large volume of data now confirms resistance to the best described drugs of the current antiviral repertoire. In the case of emerging and re-emerging viruses such as hepatitis C virus (HCV) and *Flaviviridae*-related members [Dengue virus (DNV) and West Nile virus (WNV)], one of the key scientific challenges in developing effective antiviral drugs is their high mutation rate due to the lack of efficient proofreading or mismatched repair systems, which hinder the effectiveness of the virus-enzyme inhibitors (DAAs). To help overcome these challenges with new standards of care for treatment of HCV based on DAAs, my lab has been proposing for several years to shift the current paradigm on global antiviral strategies towards the exploration of novel host-directed strategic targets. It is now well established that the hijacking of host-cell biosynthetic pathways by human enveloped viruses is a shared molecular event essential for the viral lifecycle. The next frontier is identifying the common critical host-cell pathways that are hijacked by pathogenic human viruses in order to develop broad-spectrum host-directed antivirals with novel mechanisms of action: i.e., indirect-acting antiviral agents (IAAs). The main objective of this research program is to discover new IAA strategies for treating HCV infection that could act synergistically in combination therapy with the current and upcoming standards of care involving DAAs. Our recent work published in ***PLoS Pathogens (2012)*** supports our main research hypothesis: We demonstrated that strategic manipulation of host cell SKI-1/S1P enzymatic activity in hepatoma cells by our novel protein-based inhibitor (RRLL-S) provides a means of effectively inhibiting HCV infection. The outcome of the proposed studies will lead to new insights into IAA strategies for treating *Flaviviridae* infection that could act synergistically in combination therapy with the current standards of care.

Key paper: Olmstead, A. D., Knecht, W., Lazarov, I., Dixit, S. G., and **Jean, F.** (2012) Human subtilase SKI-1/S1P is a master regulator of the HCV lifecycle and a potential host cell target for developing indirect-acting antiviral agents. ***PLoS Pathogens.*** 8(1): e1002468. Underlined in *Nature?s Science-Business exchange SciBX* **5**(6). Our paper is already listed as one of the most viewed *PLoS Pathogens* articles in the ?most views, all time? section (**5,185 article views as of October 27, 2012**). *Names of authors from my lab are underlined.*

Other papers of interest from the Jean lab in the field of protein-based therapeutics: Richer M et al., Jean F et al. (2004) *Proc. Natl. Acad. Sci. U.S.A* 101:10560-10565*;* Jean F et al. (2000) *Proc. Natl. Acad. Sci. U.S.A* 97:2864-2869; Jean F et al. (1998) *Proc. Natl. Acad. Sci. U.S.A*. 95:7293-7298

Discovering Novel Direct-Acting Antiviral Agents (DAAs) Targeting Virally Encoded Proteases



Targeting viral protease-associated replication complexes for antiviral chemotherapy: From membrane-targeted activity-based probes to novel naturally occurring protease inhibitors. Left panel: We developed and applied a novel series of membrane-anchored red-shifted fluorescent protein substrates to detect West Nile virus (WNV) NS2B/NS3 protease activity in human cells. Our study is the first to provide cellular insights into the biological and enzymatic properties of NS3, a prime target for inhibitors of WNV replication [Condotta, S. et al. and **Jean, F.** (2010) *Biological Chemistry*: Cover Illustration]. **Right panel**: The work presented in this communication demonstrates the importance of our membrane-targeted activity-based probes in studying the enzymology of complex induced-fit viral proteases such as the ER-anchored HCV NS3/4A during viral infection. [Martin, M., **Jean, F.** (2006) *Biological Chemistry*: Cover Illustration].

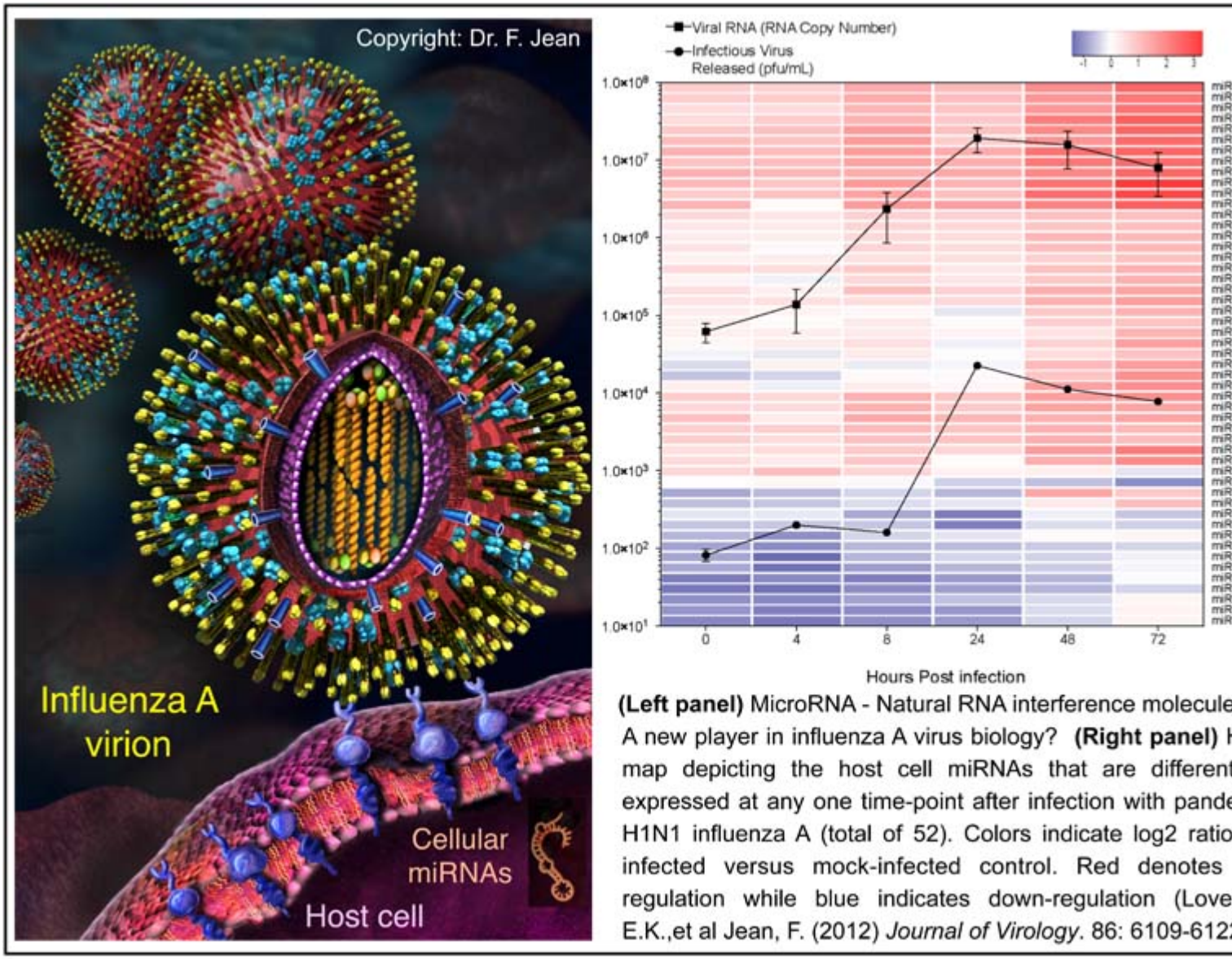
Project summary: The long-term goals of this research program targeted at virally encoded proteases are to increase our understanding of the virus host-cell interactions, and to discover how viral enzymatic pathways essential for the virus lifecycle can be interrupted. Our research program centers now on developing and evaluating new protease inhibitors of the *Flaviviridae*-encoded serine protease nonstructural (NS)3, which is essential for the viral lifecycle of hepatitis C, West Nile, and Dengue viruses. Over the last 10 years, my research in antiviral drug discovery has been punctuated by important contributions including 2 collective patents and 9

collective applications submitted to the University-Industry Liaison Office (UILO) at UBC. My team has discovered novel classes of naturally occurring direct-acting antivirals (DAAs) targeting viral proteases that are essential for the virus lifecycle of emerging and re-emerging human viruses of great concern around the world (e.g., SARS-CoV, HCV, DENV, and WNV]. One of our most exciting discoveries, novel small-molecule DAAs of the HCV NS3 protease, has been filed for a patent application and was supported by a CIHR Proof of Principle (POP) grant designed to advance discoveries towards commercializable technologies. My team has also reported the discovery of the first generation of anti-SARS agents directed at the SARS-CoV 3CL protease from marine natural products [collaborator (UBC), Dr. Andersen; **Paper of the Year 2006** (Hamill P., et al. **Jean F.** (2006) *Biological Chemistry* 387: 1063-74].

Key paper: Martin, M., Condotta, S., Fenn, J., Olmstead, A., **Jean, F.** (2011) In-cell selectivity profiling of membrane-anchored and replicase-associated hepatitis C virus NS3-4A protease reveals a common, stringent substrate recognition profile. ***Biological Chemistry***. 392: 927-35. The work presented in this communication demonstrates the importance of our membrane-targeted activity-based probes in studying the enzymology of complex induced-fit viral proteases such as the ER-anchored HCV NS3/4A during viral infection. The findings of our studies also demonstrate the potential of our experimental approaches based on membrane-targeted activity-based probes for antiviral drug screening directed at complex induced-fit membrane-bound viral proteases in the context of viral infection. For example, our fluorescent probes will greatly help the scientific research community in validating the specificity of novel anti-HCV small molecules targeted at HCV NS3/4A when tested against NS3-4A activity alone, in replication complexes, or within the course of HCV infection. *Names of authors from my lab are underlined.*

Other papers of interest from the Jean lab in the field of viral proteases as therapeutic targets: Condotta, S., et al., Jean, F. (2010) *Biological Chemistry.* 391: 549-59. **(Cover Illustration)**; Martin, M. and Jean, F. (2006) *Biological Chemistry*. 387: 1075-80. **(Cover Illustration)**; Hamill, P., et al., Jean, F. (2006) *Biological Chemistry.* 387: 1063-74 **(Awarded Paper of the Year 2006: Board of Editors of Biological Chemistry)**; Hamill, P. and Jean, F. (2005) *Biochemistry*. 44:6586-96; Richer, M., et al. Jean, F. (2004) *J. Biological Chemistry* 279:10222-10227.

Exosomal MicroRNAs as Master Regulators of Viral Infection and Blood-Based Diagnostic Biomarkers of Human Viral Diseases



(Left panel) MicroRNA - Natural RNA interference molecule A new player in influenza A virus biology? **(Right panel)** H map depicting the host cell miRNAs that are different expressed at any one time-point after infection with pande H1N1 influenza A (total of 52). Colors indicate log2 ratio infected versus mock-infected control. Red denotes regulation while blue indicates down-regulation (Love E.K.,et al Jean, F. (2012) *Journal of Virology*. 86: 6109-612

Host-cell microRNA fingerprints reveal new insights into influenza A biology. Unraveling the molecular basis of swine-origin H1N1 pandemic influenza A virus and highly pathogenic avian-origin H7N7 influenza A virus pathogenesis by microRNAome (miRNAome) analysis [see Loveday, E. K., et al., and **Jean, F.** (2012). ***Journal of Virology*** 86: 6109-6122]

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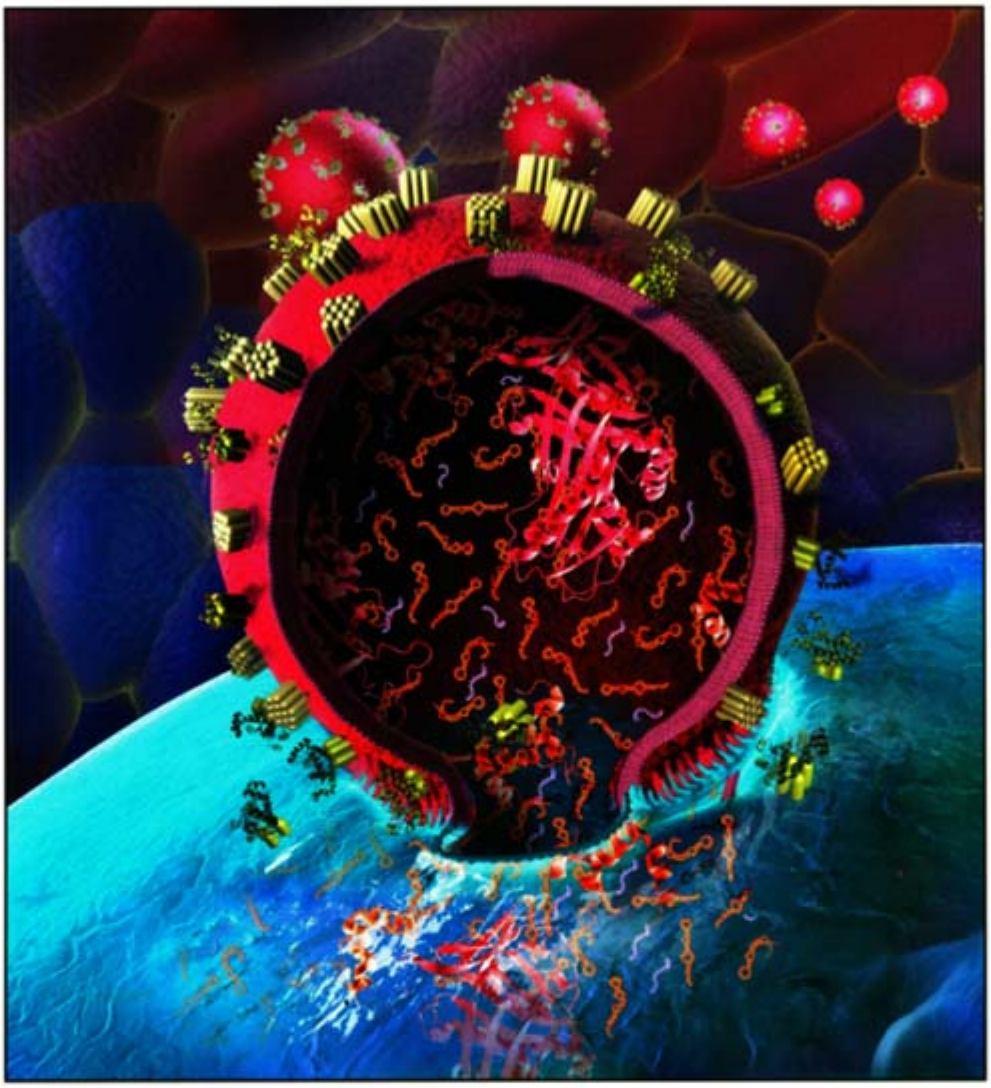

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