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Review

The HIV-1 Entry Process: A Stoichiometric View

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HIV-1 infection starts with fusion of the viral and the host cell membranes, a process mediated by the HIV-1 envelope glycoprotein trimer. The number of trimers required to complete membrane fusion, referred to as HIV-1 entry stoichiometry, remains under debate. A precise definition of HIV-1 entry stoichiometry is important as it reflects the efficacy of the viral entry process and steers the infectivity of HIV-1 virion populations. Initial estimates suggested a unanimous entry stoichiometry across HIV-1 strains while recent findings showed that HIV-1 strains can differ in entry stoichiometry. Here, we review current analyses of HIV-1 entry stoichiometry and point out future research directions to further define the interplay between entry stoichiometry, virus entry fitness, transmission, and susceptibility to antibody neutralization.

Trends

A refined understanding of the HIV entry stoichiometry emerges, indicating that most HIV-1 isolates require more than one envelope glycoprotein trimer for target cell entry.

HIV-1 strains can differ in entry stoichiometry, reflecting the divergent potential of their envelope trimers to solicit energy required for the membrane fusion process.

The HIV-1 entry stoichiometry is intimately linked with virus entry kinetics.

The HIV-1 entry stoichiometry is a contributing factor of virus infectivity.

The HIV-1 entry stoichiometry is an important parameter for the accurate determination of antibody numbers and concentrations required for virus neutralization.

The HIV-1 Entry Stoichiometry: How Many Trimers Are Required for Virus Entry?

HIV-1 enters target cells by means of envelope (Env) glycoprotein trimers embedded in the viral membrane, with each virion typically containing 5 to 15 trimers [1–7]. Each trimer consists of three heterodimers of the noncovalently associated transmembrane glycoprotein gp41 and the surface glycoprotein gp120 [8]. Gp120 contains binding sites for host cell receptor CD4 and a chemokine co-receptor, commonly CCR5 or CXCR4. Gp41 anchors the trimer in the viral membrane and mediates membrane fusion through refolding into a six-helix bundle in the final stages of the entry process [9]. The sequential steps of the HIV entry process have been extensively studied and are defined in high molecular detail [10,11]. However, the number of Env trimers and host cell receptors required to interact for productive infection (referred to as HIV-1 entry stoichiometry, T) remains under debate [6,12–20]. In this review we summarize recent findings that elucidate the HIV entry stoichiometry and discuss advantages and limitations of the different experimental approaches. We specifically emphasize the biological relevance of HIV entry stoichiometry, exemplified by functional links between entry stoichiometry, virus infectivity and entry kinetics, and antibody neutralization. We further suggest avenues for future research required to advance the understanding of the HIV entry process and its stoichiometric relations.

Determining HIV Entry Stoichiometry with Mixed Trimer Assays

A frequently applied approach to analyze HIV entry stoichiometry relies on assessing the infectivity of HIV-1 virions carrying mixed trimers composed of entry-competent and entry-deficient Env subunits (Table 1). The first comprehensive study applying this setup was reported by Yang and colleagues [12] using Env mutations with a dominant-negative phenotype, where one mutant subunit per trimer renders the trimer nonfunctional. Cotransfection of virus-producer cells with different fractions of plasmids encoding functional (wild-type, wt) and dominant-negative Env allowed the generation of virus stocks containing different compositions of mixed trimers. These mixed trimer virus stocks were analyzed for infectivity and the entry stoichiometry was retrieved by data analysis with mathematical modeling.

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Table 1. Summary of HIV-1 Entry Stoichiometry Studies Using the Mixed Trimer Approach

Study	Yang *et al.* [12]	Herrera *et al.* [14]	Klasse [13]	Magnus *et al.* [15]	Zarr and Siliciano [17]	Brandenberg *et al.* [6]
HIV-1 strain (Env)	YU2	JR-FL HxB2 3.2P	Same data as in Yang *et al.* and Herrera *et al.*	Same data as in Yang *et al.*	HXB2 YU2	SF162, NL4-3, JR-FL, RHPA, REJO, AC10, P3N, ZA110, CAP88, ZM214, BG505, various mutants thereof
Env mutation	V513E R508S/R511S	R508I/K510G			D368R R308L or R315G/L317S	V513E R508S/R511S
Target cells	Cf2Th-CD4/CCR5 cells	U87.CD4.CCR5 or U87.CD4.CXCR4 cells			Primary $CD4^+$ T cells	TZM-bl cells, primary $CD4^+$ T cells
Model	Accounting for trimers composed of wild-type Env only, strictly speaking, only modeling one trimer per virion that has 3xT Env subunits	Same as in Klasse	**(1) Liminal:** a virion is infectious if it has at least T functional trimers **(2) Incremental:** each trimer contributes equally to virion infectivity	**(1) Basic:** A virion is infectious if it has at least T trimers **(2) Segregation:** homo-trimers are preferentially built **(3) Imperfect transfection:** envelope plasmids are differently expressed **(4) Proximity:** trimers have fixed positions on the virion surface **(5) Soft threshold:** infectiousness increases with trimer numbers	Combination of liminal and incremental model as described by Klasse	Basic model described in Magnus *et al.*
Trimer numbers per virion	Not included in model	**Liminal**: constant, nine trimers per virion **Incremental**: no assumptions	**Liminal:** constant, nine trimers per virion **Incremental:** no assumptions	Discretized B-distributed trimer numbers ranging between 0 and 100 with mean 14 and variance 49, distribution defined in [15]	Simultaneous estimate of constant virion trimer number; 1 to 3 functional trimers estimated per virion	Discretized B-distributed trimer numbers ranging between 0 and 100 with strain-specific mean trimer number defined experimentally in [6]
Estimated T	**T = 1**	**T = 4 to 5** (Estimation of T was performed by Klasse)	Liminal model: **T = 4 to 5** Incremental model: NA[a]	Basic model: **T = 8** Segregation model: **T = 19** Imperfect transfection model: **T = 19** Proximity model: **T = 2** Soft threshold model: NA[a]	**T = 1 to 2**	**T = 1 to 7** (higher T's for Env ΔV1V2 and selected point mutants)

[a]NA, not applicable