

POSTER 28**3D STRUCTURE DETERMINATION OF THE HIV-1 PACKAGING SIGNAL BY HIGH-RESOLUTION MASS SPECTROMETRY**Arie Hawkins¹, Eizadora Yu², and Daniele Fabris¹¹University of Maryland, Baltimore County, Baltimore, MD; ²Sandia National Labs, Livermore, CA

The structure of the HIV-1 packaging signal (Ψ -RNA) was solved by an MS3D approach that relies on a combination of footprinting and bifunctional cross-linking coupled with mass spectrometric detection. Nuclease digestion of probed samples enables structural elucidation of inherently large and flexible substrates that are not readily amenable to the established techniques. In the case of dimerization-deficient Ψ -RNA, monofunctional footprinting revealed that the secondary structures of the four discrete stemloop domains (SL1, SL2, SL3, SL4) were largely conserved in the full-length construct. This allowed us to employ the available high-resolution coordinates of the individual stemloops as the building blocks for Ψ -RNA. Bifunctional cross-linkers provided the constraints necessary to triangulate the reciprocal positions of the various stemloops in 3D space. Modeling was accomplished by assembling *in silico* the PDB coordinates of the individual stemloops with the linker regions generated by MC-SYM. Simulated annealing and model minimization was performed using CNS.

The all-atom 3D structure of full-length Ψ -RNA maintains four stemloop domains that are closely packed in a cloverleaf morphology. This arrangement leaves the single-stranded loops and linker regions exposed and accessible to specific binding by the viral nucleocapsid (NC) protein. The availability of six exposed sites accounts for the stoichiometry observed *in vitro* for monomeric Ψ -RNA and rules out the possibility that a super-site of increased affinity may be formed. The cloverleaf morphology is stabilized by a long-range GNRA loop-receptor interaction between SL4 and SL1, which was validated by probing an *ad hoc* mutant. The observation that SL4 can be alternatively involved in loop-receptor interactions or in NC binding *in vitro* is consistent with the known propensity of GNRA tetraloops to participate in specific RNA-RNA and RNA-protein interactions. In the context of full-length Ψ -RNA, a possible switch between SL1 and NC binding may constitute the basis of a mechanism by which the Gag polyprotein could control the folding of the packaging signal and modulate its activity. Validating such mechanism *in vivo* could promote the development of therapeutic strategies aimed at disrupting the alternative binding of SL4 to SL1 or the NC-domain of Gag polyprotein.

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