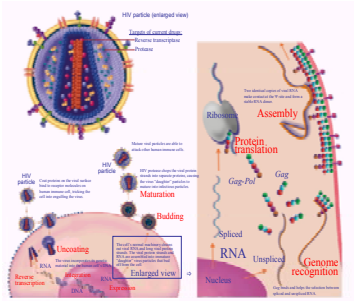
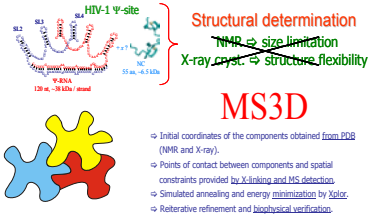
Fabris Lab

MS-based 3D (MS3D) structural investigation of protein-nucleic acids assemblies



Partial adaptation from "The Structure of Life", NIH publication No. 01-2778, National Institute of General Medical Sciences, Nov. 2000



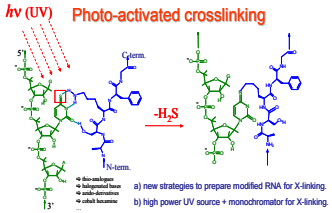
Structural determination

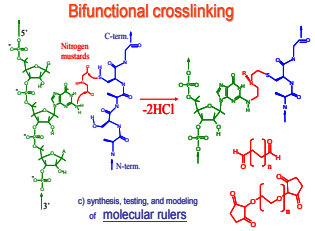
~~NMR ⇒ size limitation~~

~~X-ray cryst. ⇒ structure flexibility~~

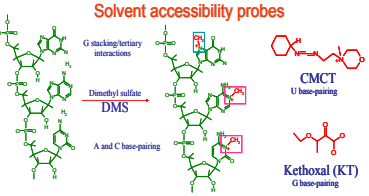
MS3D

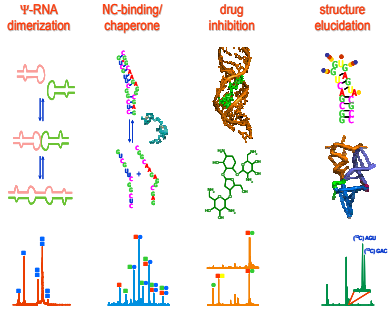
- ⇨ Initial coordinates of the components obtained from PDB (NMR and X-ray).
- ⇨ Points of contact between components and spatial constraints provided by X-linking and MS detection.
- ⇨ Simulated annealing and energy minimization by Xplor.
- ⇨ Reiterative refinement and biophysical verification.

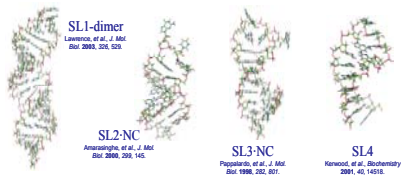




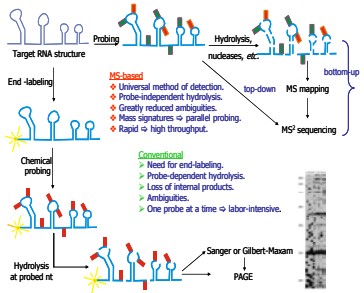
Zhang et al., J. Am. Soc. Mass Spectrom. 2006, in press.



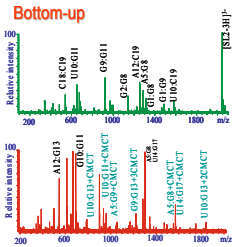


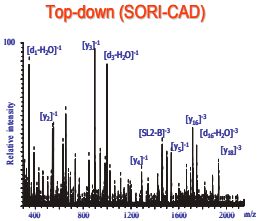


Initial assumption: the individual RNA secondary structures are sufficiently stable to be conserved in the global fold (Yusupov et al. "Crystal structure of the ribosome at 5.5 Å resolution", Science 2001, 292, 883-896).



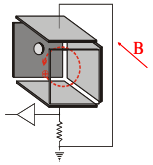
- Yu, E.; Fabris, D., Anal. Biochem 2004, 334, 356.
- Akinisiku et al., J. Mass Spectrom. 2005, 40, 1372.

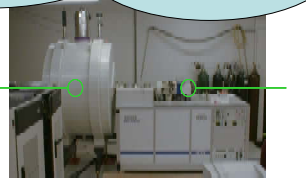


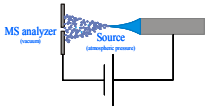


Spectra interpretation aided by MS2Links.

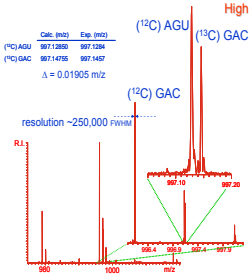
Kellersberger et al., Anal. Chem. 2004, 76, 2438.

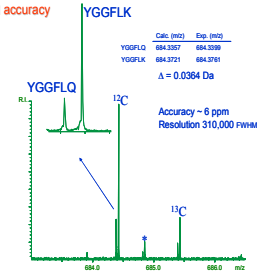






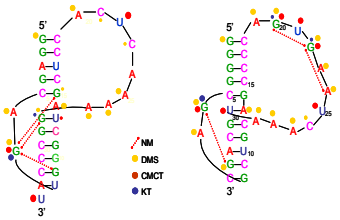
High resolution and accuracy





Kellersberger et al., Anal. Chem. 2004, 76, 3930.

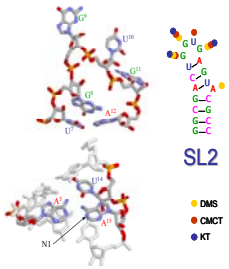
Retroviral frameshifting pseudoknots



VPK Constraints
- 34 nt-sequence
- 11 base-pairing
- 3 long-distance

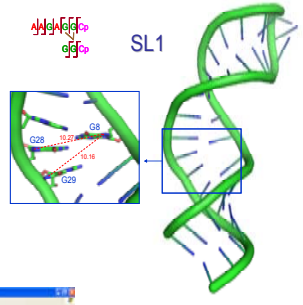
FIV-PK Constraints
- 35 nt-sequence
- 10 base-pairing
- 3 long-distance

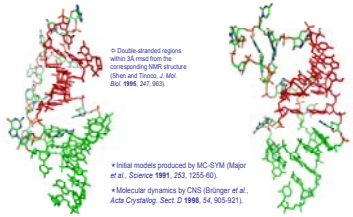
Footprinting constraints



Yu, E.; Fabris, D., J. Mol. Biol. 2003, 330, 211

Crosslinking constraints

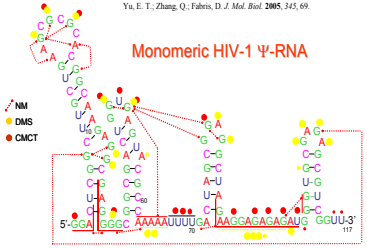


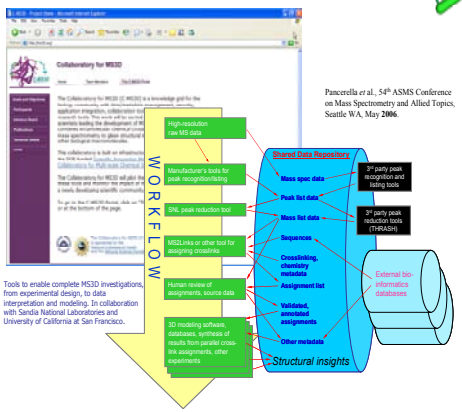


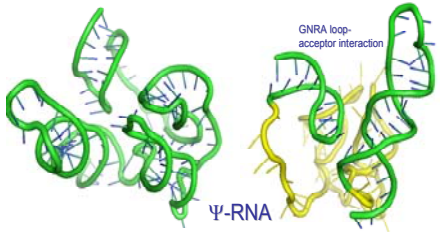
- ⇨ Double-stranded regions within 3Å rmsd from the corresponding NMR structure (Shen and Tinoco, J. Mol. Biol. 1995, 247, 963).
- ★Initial models produced by MC-SYM (Major et al., Science 1991, 253, 1255-60).
- ★Molecular dynamics by CNS (Brünger et al., Acta Crystallog. Sect. D 1998, 54, 905-921).

Yu, E. T.; Zhang, Q.; Fabris, D. J. Mol. Biol. 2005, 345, 69.

Monomeric HIV-1 Ψ-RNA





Tools to enable complete MS3D investigations, from experimental design, to data interpretation and modeling. In collaboration with Sandia National Laboratories and University of California at San Francisco.

Pancerella et al., 54th ASMS Conference on Mass Spectrometry and Allied Topics, Seattle WA, May 2006.