Advantages and Challenges in the design and application of MALDI-Cryogenic FTMS for Proteomics

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Recent reports on the use of MALDI-FTMS for peptide mass fingerprinting[1-3] have shown the strong promise of MALDI-FTMS for proteomics due to the combination of high resolution (RP > 100,000), high mass accuracy (<2 ppm), sensitivity (~1 fmol for tryptic digests), and MS^n (n > 4) capability of FTMS instruments. Automation is all that is required to modify commercial MALDI-FTMS instruments for high throughput peptide mass fingerprint analyses. However, as good as this performance is, "Best case" performance in FTMS instrumentation is far better, with RP > 8,000,000,[4] mass accuracy ~ 0.1 ppb,[5] and sensitivity in the sub-attomole regime.[6,7] Reaching these performance ranges routinely and improving them beyond their current levels will require fundamental improvements in the instrumentation.

In order to achieve such fundamental improvements, it is useful to consider where the fundamental limits of mass spectrometry arise. Resolution, sensitivity, and mass accuracy are highly dependent on the magnetic field strength, so that it is desirable to increase magnetic field if possible. These parameters are also limited by the base pressure of the instrument, which is determined (in a fully baked-out chamber) by the pumping speed. Since the cost and difficulty of building higher field magnets with sufficient homogeneity for FTMS increases dramatically with bore diameter, magnetic field and pumping speed become counteracting limits on performance, which must be balanced in any particular instrument design. Sensitivity is also limited by the (primarily Johnson) noise of the preamplifiers. These three fundamental limiting factors, magnetic field, pumping speed, and Johnson noise, become substantially different if the whole vacuum chamber, including the ICR cell, is dropped to a temperature of 4.2 K.

Below ~50 K, vacuum system walls (as well as any other surface) become cryopumping, which increases pumping speed by several orders of magnitude. Thus, if the vacuum chamber can be placed in thermal equilibrium with the liquid helium that surrounds the magnet itself, the tradeoff between magnetic field and pumping speed is eliminated, and the cold chamber can be used to decrease Johnson noise on the preamplifier by almost an order of magnitude. While desirable, construction of an instrument in this geometry requires solving some special problems, most of which were discussed in a recent publication on this topic.[8] Several points that were not discussed, however, involve certain practicalities of the design that are described here.

The crucial point of the instrument design is in developing a magnet dewar, cooled by a cryorefrigerator, which can hold the magnet stably at 4.2 K, while also allowing sufficient cooling capacity for bringing the vacuum chamber below ~50 K (or preferably lower for improved noise performance). The current sketch of this dewar is shown in Figure 1. This dewar is designed with an inner vacuum chamber that separates the inner vacuum system from the liquid helium so that the bore is at vacuum. This dewar also is designed with copper cooling brackets for the ICR vacuum chamber. The current design will have ~0.2 W of heat load plus the ~0.2-0.4 W of heat load of the ICR vacuum chamber itself.

One useful new capability of this design is that the inner bore vacuum chamber can be vented with dry helium and the ICR chamber can then be pulled out. While vented, the dewar will have an additional ~0.5 W of heat load, but this will only need to be handled for a few minutes while the ICR chamber is removed. This capability will allow the ICR chamber to be inserted/removed from the magnet while it is still charged, thus allowing the chamber to be worked on or regenerated on a monthly basis. It will also allow the cell plates and/or ion optical elements to be heated to ~80 K daily (if necessary) for a few minutes to drive the accumulated (insulating) nitrogen ice off to the in-chamber cryopumps or chamber walls. Thus, most of the inherent difficulties of use of the previous design are avoided.

The chamber also has optical access via a small (~2 mm diameter) window from the bottom of the chamber. This window, therefore, will allow photodissociation and generation of photoelectrons for electron capture dissociation in the cell.

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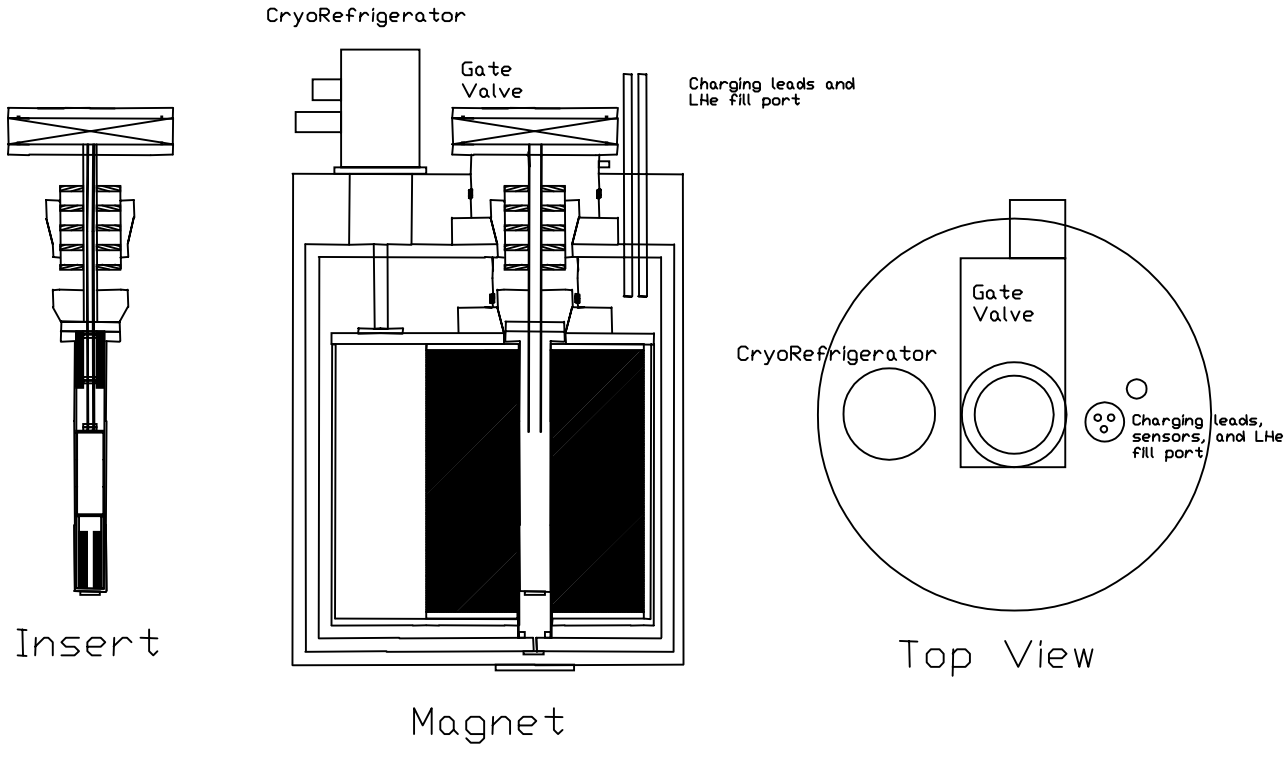

Figure 1. Sketch of the Current CryoFTMS System Design