SPATIALLY RESOLVED ANALYSIS OF AMINES USING A FLUORESCENCE MOLECULAR PROBE: MOLECULAR ANALYSIS OF IDPS. Simon J. Clemett[1], Scott Messenger[2], Kathie L. Thomas-Keprta[1], Susan J.Wentworth[1], George-Anne Robinson[3], and David S. McKay[4]; [1]Lockheed Martin, Mail Code C-23, NASA/JSC, Houston, TX 77058; email: simon.j.clemett@jsc.nasa.gov; [2]McDonnell Space Sciences Center, Washington University, St. Louis, MO 63130; [3]BayTech, Mail Code C-23,NASA-JSC, Houston, TX 77058; [4]NASA/JSC, SN, Houston, TX 77058.

Introduction: Some interplanetary dust particles (IDPs) exhibit large isotopic excesses in deuterium (^{2}H) and/or ^{15}N relative to terrestrial values. It is likely these excesses represent the partial preservation of presolar materials, formed in cold, dense molecular clouds, that predated our Solar System. The molecular carriers phase for these isotopic anomalies are believed to be organic species [1,2].

Unfortunately, while elemental and isotopic analysis of IDPs has achieved a high level of development, molecular analysis of these same samples remains a formidable challenge. This is due to the thermal fragility of organic molecules and the small sample size. Current analytical techniques such as secondary ion mass spectrometry (SIMS) produce only low ion yields for most organic species concomitant with extensive fragmentation. As a consequence very little is known either about the organic component of IDPs and it relationship to mineralogical phases present. To address the problem we are developing a procedure to spatially resolve the distribution of organic species on IDP thinsections at sub-micron resolution.

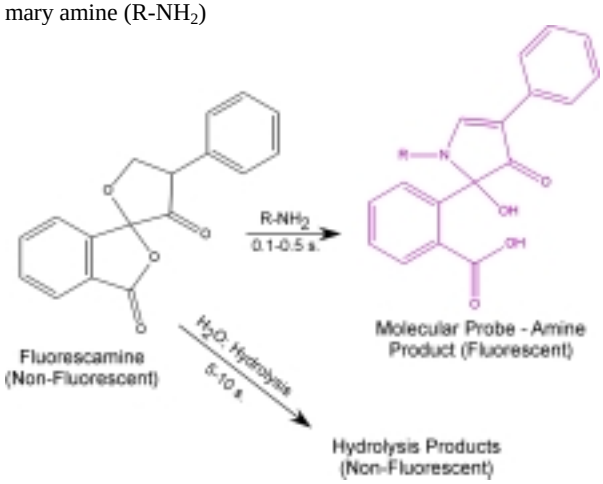
Concept: Our approach is to target specific organic monofunctional groups and tag them with a fluorescent molecular probe. Once a sample surface has been tagged the spatial distribution of those monofunctional groups can be determined by fluorescence microscopy. This same surface can then subsequently be analyzed by two-step laser microprobe mass spectrometry (μL^2MS) to determine the specific molecular species tagged.

Since a single fluorphor molecule is typically capable of being consecutively cycled through the fluorescence "excitation-emisson" sequence many times, that is it has a low-photobleaching quantum yield, single molecule detection can routinely be achieved by fluorescence microscopy [3]. The spatial resolution limit is determined by the wavelength of the light emission from the fluorophor and is typically submicron.

Because most fluorphors are conjugated and/or aromatic species they are also ideally suited to analysis by μL^2MS. μL^2MS is a powerful technique for spatially resolved analysis of sub-attomole concentrations of complex aromatic molecules [4]. By using a fluorescent molecular probe to selectively tag particular organic molecules, species ordinarily not amenable to μL^2MS analysis (i.e. non-aromatic species such as amino acids) become readily detectable. Moreover, since it is the fluorphor of the molecular probe that undergoes multiphoton resonant ionization the photoionization cross sections for tagged molecules are approximately constant, allowing for direct qunatitation of the results from μL^2MS analysis

Method: The molecular probe we have chosen is 4-phenylspiro[furan-2(3H),1'-phthalan] 3,3'-dione, hence forth referred to as fluorescamine. This is a novel reagent for the analysis of primary amines, see Figure 1 [5]. Fluorescamine itself is not natively fluorescent, but in aqueous solution (pH > 8) undergoes a rapid reaction with primary amines (R-NH_2) to yield a highly fluorescent moiety (380 nm excitation; 480 nm emission). The reaction half-life for derivatization is a ~ 0.1-0.5 seconds at room temperature. Unreacted or excess reagent is quickly deactivated by hydrolysis, for which the reaction half-life is on the order of 5-10 seconds. The hydrolysis products, like the native reagent, are non-fluorescent.

Figure 1: Fluorescamine derivatization reaction with a primary amine (R-NH_2)



Experimental: As proof of concept Murchison (CM2) was choosen as a model substrate. Figure 2 show a freshly microtomed section of Murchison (CM2) in a N-free epoxy bullet. The exposed face was lowered onto a drop of water, buffered by carbonate to pH 8.6. To this ~20 µl of 10^{-3} M. fluorescamine solution in acetone was added by a glass hypodermic syringe with a stainless steel needle. The derivatization was allowed to proceed for 10 seconds after which the sample was removed and dryed in a stream of dry N_2.

The distribution of primary amines was then determined using an Olympus BX20 fluorescent microscope equipped with an image intensifier and CCD array. Figure 3 shows this amine distribution overlayed on a backscattered electron (BSE) image obtained by a JEOL 6340F Field Emission scanning electron microscope (FESEM). Correlation in the amine distrubtion with elemental abundances are decernible in energy dispersive x-ray (EDX) maps for Si, Fe, O & Si,

see Figure 4. The amine distribution is correlated with Si and O but anti-correlated with Fe and S.

Figure 2: Murchison (CM2) particle microtomed in N-free epoxy block

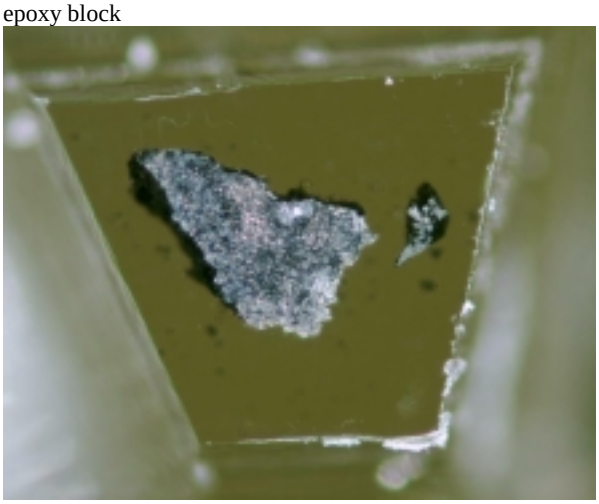
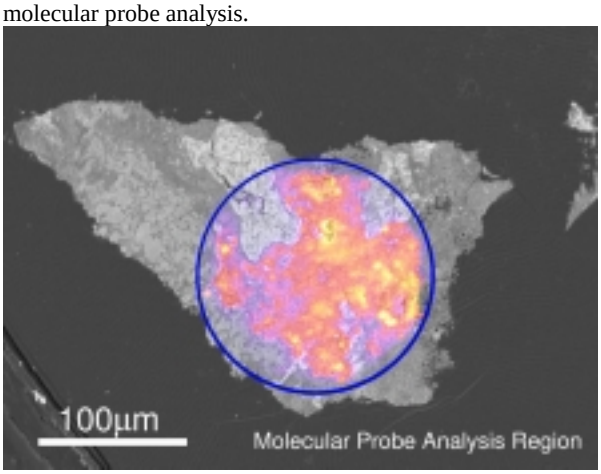


Figure 3: BSE image of Murchison (CM2) block overlayed with the amine distribution map as determined by fluorescent molecular probe analysis.



In a separate experiment the amino acids valine and isoleucine were spiked at meteoritic concentrations into a pulverized basalt matrix and derivatized by fluorescamine. The fluorescamine derivatized amino acids were then analyzed by μL^2MS as shown in Figure 5. Both the parent ion peaks of the fluorescamine tagged valine (377 amu) and isoleucine (391 amu) are observered along with the accompanying [-CO_2H] loss peaks (333 & 347 amu respectively).

Conclusions: We report the first measurements on the spatially resolved molecular distribution of primary amine in a meteoritic sample. The technique is fast, non-invasive, and mineralogically non-destructive and can be used in conjuction with elemental and isotopic measurements. The molecular analysis of IDPs using specific fluorescent molecular probes is feasible and prelimnary experiments suggest that it has the potential to help resolve whether the carrier phase(s) for isotopic anomalies in IDP is(are) organic in nature. The usefulness of the technique should be applicable to a wide range of samples in the fields of cosmochemistry and astrobiology.

Figure 4: Element distriburion maps determined by FESEM-EDX for Si, Fe, O & S

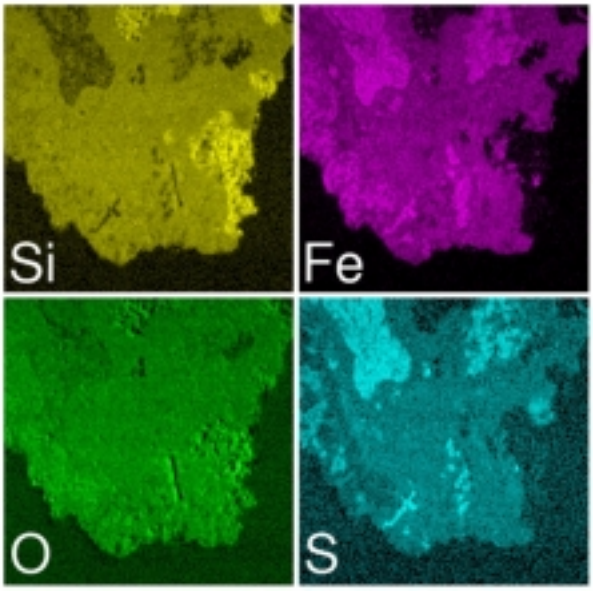
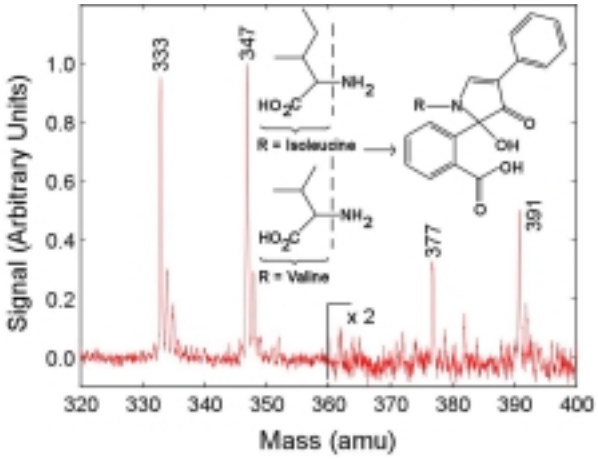


Figure 5: Laser microprobe spectrum of the fluorescamine derivatized amino acids valine & isoleucine



Acknowledgements: Prof. Richard N. Zare, Xavier D.F Chillier and the ZareLab.

References: [1] Messenger, S. & Walker, R.M. (1996*) AIP Conf. Proc.* **402**, 545-564; [2] Keller, L. P., et al. (1997) *LPSC* **XXVIII**, 707-708; [3] Eggeling, C., et. al. (1998) Applied Fluorescence in Chemistry, Biology and Medicine, Springer-Verlag; [4] Clemett, S.J. & Zare, R.N. (1997) *IAU Symposium* **178**, 305-320; [5] Udenfriend, S., et al. (1972) *Science*, **178**, 871-872.