DETECTION OF BIOLOGICAL MOLECULES VIA MOLECULARLY IMPRINTED POLYMERS COUPLED WITH SURFACE PLASMON RESONANCE. Lance M. Baird[1], Scott M. Levin[2], Kelly A. Van Houten[2], Robert S. Pilato[2], George M. Murray[2], Noam R. Izenberg[2] [1]lance.baird@jhuapl.edu [2]Johns Hopkins University Applied Physics Laboratory, 11100 Johns Hopkins Road, Laurel, MD 20723

Introduction: Molecularly Imprinted Polymer (MIP) - based sensors are promising candidates for a variety of in-situ planetary astrobiological and geochemical mission profiles. The goal of this project is to develop molecularly imprinted polymer (MIP) coatings for surface plasmon resonance (SPR) sensors that will be specific for amino acids, peptides and proteins[1]. The coatings prepared in this project will benefit from recently developed technology that allows preparation of MIPs that are water soluble and processable. The system will be developed to obtain low limits of detection with a low probability of false negatives or false positives. By basing the system on the commercially available SensíQ™ surface plasmon resonance instrument the analysis process will be rapid, automated and low cost.

Background: Molecular imprinting is a technique for making a selective binding site for a specific chemical. The technique involves building a polymeric scaffold of molecular complements containing the target molecule. Subsequent removal of the target leaves a cavity with a structural "memory" of the target (Figure 1).

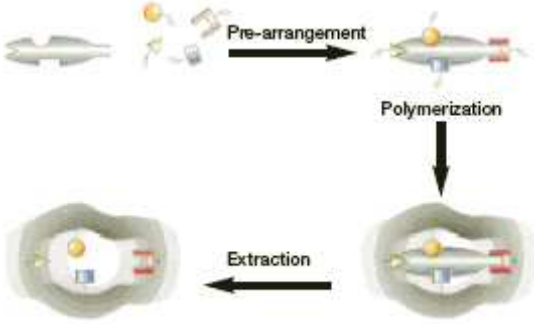

Figure 1. Process of molecular imprinting.

To build the polymer framework for the MIP, Reversible Addition-Fragmentation Transfer (RAFT) polymerization was used. RAFT polymerizations are a controlled method of living radical polymerization that uses dithioesters to mediate the polymerization in a reversible chain-transfer process. RAFT polymers generally have low polydispersity and can be highly functionalized. This polymerization method is compatible with a wide variety of monomers and allows for the production of complex architectures, including star polymers. RAFT polymers have dithioester terminal groups that can be cleaved to generate free thiols. Hence, these polymers are ideally suited for gold binding. This is particularly important to this study since the substrate for surface plasmon resonance (SPR) is a gold film.

SPR is a powerful tool used to characterize biomolecular interactions. SensíQ™ (Nomadics, Inc.) is a dualchannel, semi-automated SPR system which utilizes advanced microfluidics, and state-of-the-art data analysis tools to provide kinetic, affinity, and concentration data (Figure 2).

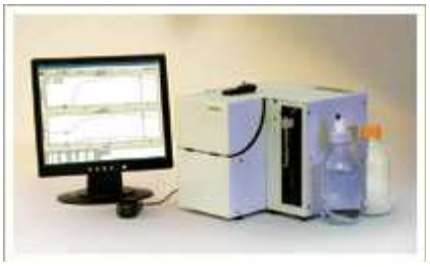

Figure 2. SensíQ™ SPR instrument.

One limitation to SPR is that direct detection of low molecular weight astrobiological biomarkers (<500 Da) using SPR is only possible in the molar to millimolar range. Such high analyte concentrations are well above those of interest to this program. To combat this issue, the biomarker can be captured by the SPR sensor and then coupled to a secondary molecular recognition event with a high molecular weight species (10 kDa – 1000 kDa). This serves to amplify the signal from the bound analyte, and makes it possible to detect biomarkers of low molecular weight.

The overall goal of this project is to detect biomarkers and biosigns for astrobiological planetary and space environment applications. Biopolymers such as proteins, RNA, and DNA degrade on the geological time scale, especially under harsh conditions. However, the respective breakdown products, amino acids and nucleobases should be readily available for detection given their geological longevity. We are preparing MIPs for amino acids and biogenic amines.

Results and discussion: In our detection system we coupled a MIP with SPR to generate the signal necessary to detect low molecular weight biogenic amines of astrobiological importance. The amine of interest was templated to the surface of the SPR sensor. After templating the sensor, a water soluble polymer imprinted for the amine was passed over the sensor. The polymer then bound to the amine. This binding amplified the SPR response of the biological molecule attached to the sensor (Figure 3).

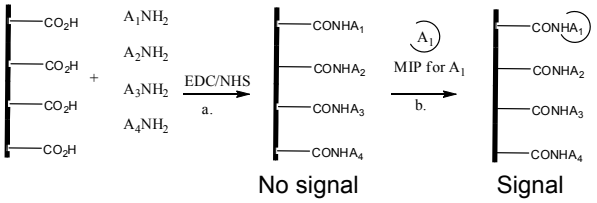

Figure 3. Scheme detailing the detection process.

The MIP was created with a water soluble 3-arm RAFT star copolymer with trimethylolpropane triacrylate (TMPTA) as the core. The polymer was composed of vinyl benzoic acid and vinyl sulfonyl styrene monomers. These monomers where chosen because vinyl sulfonyl styrene aids in making the polymer water soluble, and vinyl benzoic acid creates sites for crosslinking (Figure 4).

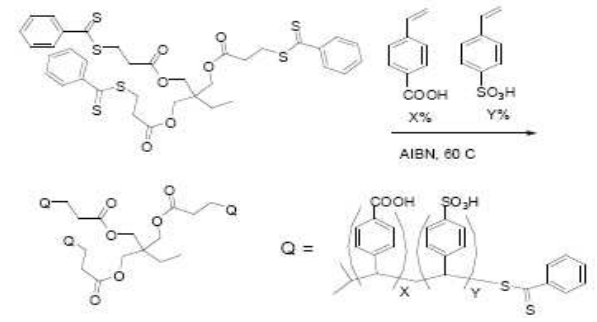

Figure 4. Raft polymerization.

Biotin (vitamin H or B_7) was chosen to imprint the polymer due to its biological importance as a cofactor in essential metabolic reactions, and because its incredibly strong binding affinity with streptavidin ($K_d = 10^{-15}$ M) can be used to compare the MIP's own binding efficiency. The imprinting process was carried out by crosslikning biotin methyl ester into the polymer via an EDC/NHS coupling reaction with 1,4-diaminobutane. The imprint molecule (biotin methyl ester) was removed by dialysis and CH_3CN washings and the polymer was lyophilized (Figure 5).

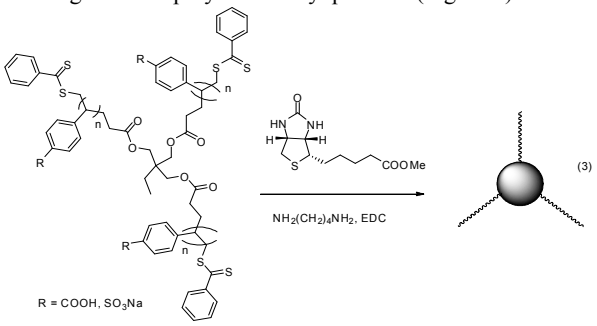

Figure 5. Imprinting process

As a crude way to measure the MIP's binding affinity for biotin, streptavidin was passed over the surface of a MIP bound SPR sensor. As can be seen in the figure 6, the MIP was displaced by the streptavidin, indicated by the curves in positions 1 and 2. In addition, the signal was amplified, 600 units higher than that of the MIP, by the binding of streptavidin. This signal amplification is due to streptavidin's high molecular weight (60KDa) in comparison to the MIP (~15-30KDa).

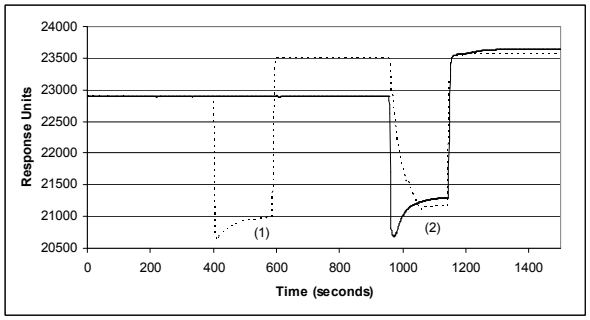

Figure 6. SPR sensogram detailing the competitive binding of streptavidin with a MIP. Reference channel (——); Signal channel (- --). Step 1 (400-600sec) and Step 2 (975-1175sec) display MP displacement. The reference channel was untreated with streptavidin in step 1.

Future Work: As can be seen in the studies above, we have developed and are continuing to develop methods for the detection of amines of astrobiological interest. Once these amines are detected, we propose to study the origin of the amines also using SPR. To this end we are preparing synthetic protein mimics that once bound to an SPR sensor would serve as substrates for extracellular enzymes. Many bacteria (including the eubacteria, and archae) as well as the protista and fungi are known to excrete extracellular enzymes that are expressed in the presence of complex carbon and nitrogen sources. These enzymes aid in the initial metabolic processing of nitrogen and carbon sources to large or complex to enter the cell.

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References:

[1] Izenberg e. al. (2006) Lun. Planet. Sci. XXXVII. [2] Wulff, et. al. (1990) *J. Liq. Chromatogr. 13*, 2987. [3] Chiefari et. al. (1998). *Macromolecules, 31*, 5559. [4] Southard et. al. (2006) *Macromolecules, 40*, 1395.