AFFINITY RESINS FOR BIOMARKER AMINE DETECTION.  Kelly A. Van Houten1,2, Robert S. Pilato2, George M. Murray2, Noam R. Izenberg2 1kelly.van.houten@jhuapl.edu 2Johns Hopkins University Applied Physics Laboratory, 11100 Johns Hopkins Road, Laurel, MD 20723

Introduction:  Molecular 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 newly 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 negative or false positives.  By basing the system on the commercially available SensiQ™ 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).

To build the polymer framework for the MIP, Reversible Addition-Fragmentation Transfer (RAFT) polymerization will be 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.  SensiQ™ (Nomadics, Inc.) is a dual-channel, 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).  SPR is amenable to multiplexing and miniaturization and hence is ideally suited for future astrobiological missions.

Figure 2. SensiQ™ SPR instrument.

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:  We have prepared water-soluble linear polymers and 3-arm star polymers using RAFT.  The core of the star was trimethylolpropane triacrylate (TMPTA) that was converted into a dithioester by addition of dithiobenzoic acid (Figure 3).  The polymerization used 4-vinylbenzoic acid (4-VBA) and 4-styrene sulfonic acid (4-SSA) as the monomers (figure 4).

Figure 3. Preparation of RAFT core.
The dithioester groups in these polymers were cleaved and the free thiol containing polymers were purified by dialysis (1000 MWCO) against water. In order to imprint these polymers, a crosslinkable group, N-(3-Aminopropyl)methacrylamide HCl (APMA) was coupled to the COOH groups via EDC/NHS coupling (Figure 5).

Biogenic amines, amino acids or fluorescently-labeled amino acids were added to the polymer, followed crosslinking (Figure 6). The templated amine was removed from the crosslinked star polymer by dialysis. The MIPS imprinted for fluorescently labeled biogenic amines, cadaverine. Cadaverine is the anaerobic breakdown product of cadaverine. Cadaverine is the anaerobic breakdown product or arginine. The presence of biotin groups on the surface of the sensor was confirmed by binding to streptavidin (Figure 7).

**Figure 5.** Addition of crosslinker.

**Figure 4.** RAFT polymerization.

**Figure 6.** Crosslinking of star MIP with an amine or amino acid.

The COOH groups were coupled to pentylamine biotin (Pierce) via 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC)/ N-Hydroxysuccinimide (NHS) coupling. Pentylamine biotin is the biotinylated derivative of the biogenic amine, cadaverine. Cadaverine is the anaerobic breakdown product or arginine.

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References: