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Surface Analysis: From Single Crystals to Biomaterials

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Abstract

Surfaces and interfaces play a critical role in material performance in many applications including catalysis, biomaterials, microelectronics, tribology and adhesion. Characterizing the important surfaces and interfaces involved in each application may present different challenges, but the approach to investigating them often is rather similar. Specialized instrumentation is typically used to probe the surface region of a material and often times it is required to develop new instrumentation and data analysis methods to obtain the desired information. It is usually best to use multiple experimental techniques, often coupled with theoretical calculations and simulations, to gain a more complete understanding of the surface and interface regions. Careful handling and preparation of the samples is required so the surface is not altered during these processes as well as during analysis. Using model samples with well-defined surface structures and compositions can provide information about fundamental processes as well as help develop the analytical tools and methodology needed to characterize complex surfaces and interfaces. Thus, the expertise and experience a surface analyst acquires in one field can be readily applied to other fields, even when those fields are significantly differently (e.g., biomaterials and microelectronics). This has resulted in surface analysts moving rather easily between different research and application areas. As one example my career path of small molecule chemisorption and reactivity on single crystals to industrial catalysis to biomedical surface science is presented in this manuscript.

Introduction

The tool chest of a surface scientist provides an excellent foundation for moving into a wide range of different research and application fields since it is the surface of a material that interfaces with its surrounding environment and its surface interactions with that environment plays a key role in determining the material's performance[1–3]. Different types of surfaces are combined to form a wide range of interfaces such as solid/gas, solid/liquid, solid/solid, liquid/liquid and liquid/gas. Although studies often just focus on one particular surface in an interface, it is usually best to consider the surface regions on both sides of the interface. Some interfaces (e.g., solid/vacuum) are straightforward to characterize while other interfaces (e.g., solid/liquid or buried solid/solid) can offer special challenges to characterize. However, regardless of the interface to be characterized, the surfaces involved represent regions that are only a few atoms or molecules thick[4]. All surface scientists and analysts are well aware of the special instrumentation and methodology required to study surfaces and interfaces[5]. It is the experience, knowledge and approaches for how to selectively characterize the small amount of matter present in the

surface region along with understanding of how to handle and prepare samples that preserve the highly reactive surface regions in their native state for analysis is what allow surface scientists to pursue important research and development problems in fields such as catalysis, adhesion, biomaterials, microelectronics, corrosion and tribology. In fact, the tools of the surface scientist allow us to move rather easily between rather different fields (e.g., catalysis to biomaterials or biomaterials to microelectronics).

Many surface scientists started their research careers by investigating the chemisorption and reactivity of molecules with single crystal surfaces as part of their Ph.D. studies. A few of these researchers ended up spending most of their careers in this area, but most of us went on to work in different areas. A common destination for surface scientists has been the field of catalysis given the importance of surfaces in catalytic reactions, but other fields such as microelectronics have also been a choice of many. In recent years fields such as biomaterials and clean energy have become increasing popular destinations for surface scientists. Given my career path of small molecule chemisorption and reactivity on single crystals to industrial catalysis to biomedical surface science, I will use that as one example on how surface scientists evolve during their careers. This adventure has included the design and construction of novel instrumentation, the adaptation of surface analysis techniques to emerging research areas and the development of new experimental protocols and data analysis methods. However, the unifying theme throughout this journey has been that the composition and structure in the surface region of a material—the interface between that material and its environment—mediates its performance in a variety of applications (catalysis, microelectronics, adhesion, biomaterials, etc.)[1, 2, 6]. Thus, the more we understand about the surface structure and composition of a material, along with the reactions that occur on its surface, the better understanding we will have of the performance of that material[7].

Small Molecule Chemisorption and Reactivity on Single Crystal Surfaces

Thanks to the space program, ultra-high vacuum (UHV) systems and instrumentation became widely available in the 1960s and 1970s, providing a great boost to surface science research. Even with the advent of the commercial systems for surface science studies during this period, custom built UHV chambers as well as home built electronics and instrument components were still commonly used (see the schematic drawing in Figure 1 for an example of a UHV system used for single crystal studies). Single crystals are ideal substrates for surface science studies[8]. Using UHV systems allowed single crystals to be prepared with well-defined, well-characterized surface structures and chemistries. Small gas molecules could then be easily dosed onto the single crystal surfaces, allowing their adsorption, desorption and reaction behaviors to be investigated with techniques such as low-energy electron diffraction (LEED), Auger electron spectroscopy (AES), x-ray photoelectron spectroscopy (XPS) and thermal desorption spectroscopy (TDS). Given the limited number of single crystal surfaces that had been investigated up to this time, there were a wide range of options to study. Some researchers chose to focus on a single adsorbate on one particular crystal face (e.g., CO on Ni(111)[9]). Others chose to study a wider range of molecules (H₂, CO, O₂, NO, C₂H₄, etc.) and surfaces (Pt, Rh, Au, Ni, etc.)[10, 11]. For the type of surface, both different crystal faces of the same metal or similar crystal faces on a

range of metals were investigated. Both approaches have their benefits. Studies of a single adsorbate system allowed the research to focus on determining with high accuracy specific details (binding site, desorption energy, etc.) of that particular adsorbate system, while studies of a wide range of adsorbates and surfaces allowed trends in the adsorbate-surface interactions to be determined.

Many of the themes present in single crystal studies are directly applicable to studies to other areas of surface analysis. These themes include multi-technique analysis[10, 11], the relationship of surface properties (structure, chemistry, etc.) to material performance (catalytic activity, biocompatibility, etc.)[12], the use of model systems to develop the tools and methodology for studying more complex systems (i.e., the “reductionist” approach)[6], and the development of novel instrumentation (specialized UHV systems, new analysis techniques, etc.)[13]. Given the small amount of material present in a surface layer coupled with the specialized methods and techniques required to specifically probe surfaces[5], it is extremely rare when one technique can characterize the surface structure and chemistry in sufficient detail. Thus, to gain a more comprehensive understanding of surfaces, complementary analysis with multiple techniques is required. This multi-technique analysis can include multiple different experimental techniques (XPS, LEED, etc.) as well as theoretical calculations and simulations (e.g., molecular dynamics)[14]. The state of the surface can have significant effects on the material performance. For catalytic materials the oxidation state and coordination number of surface atoms, as well as the presence of promoters and other molecular species can all affect catalytic activity and selectively[15]. For biomedical applications the surface chemistry and structure can affect the types and amounts of proteins adsorbed from the biological environment[16]. The well-defined surface chemistry and structure of single crystals make excellent model systems for developing advanced analysis methods as well as providing detailed information about fundamental surface properties such as adsorbate structure and energetics[4]. Single crystals also provide an easy means for systematically varying the type (e.g., Rh vs. Pt), structure (e.g., 111 vs. 100) and coordination number (e.g., low index plane vs. step or kink) of atoms present at the surface[4]. To bring all these themes together and develop surface property/relationships often requires the design and construction of novel instrumentation, such as systems that combine high pressure catalytic reaction chambers with UHV analysis chambers[13].

As example of these early single crystal studies were the experiments done to examine the chemisorption and reactivity of small molecules on Rh surfaces[10, 11, 17]. The molecules investigated included H₂, O₂, CO, CO₂, NO, C₂H₂ and C₂H₄. The Rh surfaces investigated included Rh(111), Rh(100), Rh(331) and Rh(775). LEED, AES and TDS were used to determine the surface structures, coverages, disordering transitions, desorption temperatures, displacement characteristics and decomposition reactions for each of these molecules. The influence of step atoms on these surface properties was investigated by comparing the results from the Rh(331) (40% step density) and Rh(775) (17% step density) surfaces to the low index Rh(111) surface (see Figure 2 for structures of these three surfaces). The presence of the step atoms did influence the adsorbate properties, with the degree of influence increasing with increasing step density (i.e., the influence of steps on Rh surface chemistry increases as Rh(111) < Rh(775) < Rh(331)). Although some of the adsorbate structures observed on the Rh(111) surface were also observed on the (111) terraces of Rh(775) and Rh(331) surfaces,

in general the long range adsorbate ordering was decreased on the stepped surfaces and some new adsorbate structures were observed on the Rh(331) surface. In addition some dissociation of CO was observed on the two Rh stepped surfaces. The chemisorption of these small molecules on the low index Rh surfaces were similar to results observed for low index Ir and Pt surfaces. In contrast, there were some differences observed among Rh, Ir and Pt for stepped surfaces. The relationship of CO hydrogenation at 6 atmospheres to Rh surface chemistry was investigated[12]. Metallic Rh surfaces are mainly produced methane (90 wt%), but pre-oxidation of the Rh surface resulted in the appearance of oxygenated products (methanol, ethanol and acetaldehyde), as well as a significantly increase in the initial reaction rates and a significant decrease in the methanation activation energy.

Surface Analysis and Industrial Catalysis

Heterogeneous catalysts used in industrial applications are significantly more complex than the single crystal surfaces discussed in the previous section[15]. A typical heterogeneous catalyst used in petroleum refining has nanoparticles comprised of one or more elements and supported on a high surface area oxide. These supported nanoparticle catalysts can have a wide range of compositions. For example, Co/Mo or Ni/W based nanoparticles can be used for removing heteroatoms such as S and N from crude oil, while Pt based nanoparticles can be used for reforming reactions. Typical oxide supports for these catalysts include alumina, silica, zeolites and titania. How these catalysts are activated can have a significant effect on their activity and selectivity. Often times the final step in the preparation of a heterogeneous catalyst involves calcination, or heating in air[18]. This typically produces leaves the supported nanoparticles in an oxide form. The oxide nanoparticles are then subjected to a series of activation steps (reduction, sulfidation, etc.) in the catalytic reactor to produce the working catalyst[19, 20]. It is essential to characterize the chemical state and structure of the working catalyst. This requires specialized instrumentation so these supported catalysts can be prepared under well-defined conditions (e.g., reduced in H₂ at a given temperature and pressure) and then transferred directly into UHV for surface analysis[20, 21]. Since supported nanoparticles in both metallic and sulfided states are highly reactive and will quickly oxidize when exposed even briefly to air at room temperature, special care must be taken to avoid any exposure to the atmosphere when the transfer into UHV is made[21].

An example of a catalyst treatment system designed to rapidly transfer catalysts from one atmosphere and high temperatures into UHV for surface characterization with XPS is shown in Figure 3. The unique aspects of this system were the ability to treat catalysts under a variety of different conditions (oxidizing, reducing, sulfiding, etc.) without cross contamination, the rapid transfer of the samples into UHV and the trolley system used to move the samples between various stations (sample inlet, sample storage, reactors, XPS analysis, etc.). The XPS Co 2p spectra from a Co/Al₂O₃ catalyst after sequential calcination (air), reduction (H₂), sulfidation (1% H₂S/H₂) and recalcination (air) at 480°C for one hour are shown in Figure 4. These spectra show the presence of several Co species on Al₂O₃ support. Co₃O₄ particles that are reduced to metallic Co at 480°C in H₂, a Co²⁺ species that is converted to Co₉S₈ at 480°C in 1% H₂S/H₂, and CoAl₂O₄-like species that is neither reduced or sulfided at 480°C. Recalcination of the sulfided catalyst resulted in the formation

of CoSO_4 . XPS S 2p peaks were only detected from the sulfided and recalcined samples, with binding energies consistent with Co_9S_8 (162.5 eV) and CoSO_4 (170 eV), respectively.

The catalyst treatment system described in the previous paragraph was used to determine the relationship between the surface properties of supported catalysts and their catalytic activities. One example was a series of Co catalysts supported on SiO_2 , TiO_2 , Al_2O_3 and K- Al_2O_3 . [22] In addition to XPS analysis of the calcined and reduced catalysts, complementary analysis with temperature programmed reduction (TPR) and x-ray diffraction (XRD) experiments were also done. The Co species detected on the calcined catalysts included Co_3O_4 , Co^{2+} species, and CoAl_2O_4 . In one atmosphere of flowing H_2 the Co_3O_4 particles could be reduced to metallic Co at temperatures below 425°C. Some of the Co^{2+} species could be reduced to metallic Co at temperatures between 425 and 700°C. Reduction of CoAl_2O_4 required temperatures above 850°C. Differences in the amounts, types and reducibilities of the Co species present on the different supports were important in determining the CO hydrogenation of these catalysts. In particular the amount of metallic Co detected by XPS after H_2 reduction at 480°C correlated directly to the catalyst's activity in a mixture of $3\text{H}_2:\text{CO}$ at 10 atmospheres.

In addition to characterizing the chemical state of supported particles it is also important to characterize their size, as percent of surface atoms in a particle increases significantly with decreasing particle size [23]. Since the surface atoms are the ones that are the most active, quantifying the particle size or number of surface atoms is a key parameter for determining and comparing catalytic activity performance for a series of catalysts since catalytic activity is typically normalized per surface atom [4]. Due its surface sensitivity XPS can be used to determine the size of supported nanoparticles [24]. XPS was used to measure the size of Co nanoparticles on a silica support with different surface areas after initial impregnation, calcination, reduction and sulfidation [18–20]. The results are summarized in Table 1. The Co nitrate species present after initial impregnation and drying is highly dispersed. Upon calcination the Co sinters into Co_3O_4 particles. Reduction of Co_3O_4 to metallic Co significantly decreases the Co particle size due to removal of the oxygen atoms. Sulfidation of metallic Co to Co_9S_8 increases the Co particle size due to addition of the sulfur atoms. Validation of the XPS particle size calculations was done by measuring the Co_3O_4 particle size with XRD line broadening and TEM image analysis, as well as the metallic Co particle size with TEM image analysis. The good agreement of the particle size determination by the different techniques show that using the Kerkhoff and Moulijn method [24] for XPS determination of supported particle size works well. However, it is limited to supports with high surface areas ($>200 \text{ m}^2/\text{cm}^3$) and only one type of supported particle (e.g., Co_3O_4). Other methods have been developed for characterizing multicomponent unsupported nanoparticles and multicomponent nanoparticles on low-surface area supports [25].

As described above catalyst treatment systems directly connected to XPS instrumentation can provide detailed information about the chemical state and structure of catalysts. However, even with direct transfer from a reactor at high pressure and temperature into the UHV system the catalyst is still not being characterized directly under working conditions. This requires a method that can provide chemical state and structure information about the catalyst while the catalysts are at high pressures and temperatures. X-ray absorption

spectroscopy (XAS) is a technique that can provide such information[26]. The position and shape of the absorption edge can be used to characterize the chemical state of supported nanoparticles while the extended region above the absorption edge can provide information about the structure of the supported nanoparticles. When combined with the intense and tune-able x-rays produced by synchrotron radiation, data can be acquired rapidly from catalysts under working conditions[27]. A novel reaction chamber developed for these *in situ* or *operando* XAS spectroscopy studies is shown in Figure 5. When this reactor was developed in the early 1980s it provided some of the first real time *operando* XAS experimental results. Because the absorption edge spectra could be acquired rapidly (seconds) and the different chemical states each had unique absorption edge spectra, it allowed the reduction and sulfidation profiles of catalysts to be directly examined (see insert in Figure 5). For example, the first conclusive evidence for direct conversion of a metal oxide to a metal sulfide without going through a metallic intermediate was obtained with this instrumentation[19, 27]. This finding had important implications for the activation of hydrodesulfurization (HDS) catalysts. Also, in this same time period a reactor was built to characterize working HDS catalysts with XAS[28]. Now *operando* XAS instruments are widely used, both with synchrotron and laboratory x-ray sources.

Biomedical Surface Analysis

Surface analysis of catalysts and surface analysis of biomedical devices have many of the same concepts and approaches, but there are several important differences. For example, flowing crude oil over an HDS catalyst and flowing blood through a synthetic vascular graft both involve complex fluids contacting the surface of a solid material. In both cases it is important to use a complementary, multi-technique approach to characterize the surface structure and composition of the catalytic or biomedical material contacting the complex fluid and correlate their surface properties to their catalytic or biologic performance. However, the objectives for the processes are different. The objective for crude oil refining is for it to be changed by selectively undergoing reactions with the catalyst (e.g., removal of heteroatoms such as sulfur)[15]. In contrast, the objective for blood is for it not to be changed (e.g., avoid activation of platelets) upon contact with the vascular graft[29].

Crude oil contains many different chemical species, but most can be grouped into related structures and reactivities. This allows the reductionist approach to be applied for determining the overall reaction network and rates. That is, the reaction characteristics (rates, energetics, etc.) of representative model compounds can be determined individually and then combined to fully model the entire set of reactions occurring in a given catalytic reaction[30]. The biological environment has several important differences from the catalytic environment[29]. First, it contains a range of different species (proteins, lipids, cells, etc.) that each have a wide range of structures and functions. Second, the reaction network in biological environment is far more interactive and interconnected than the catalytic environment. While some insights can be gained by studying the interaction of a surface with a single protein in a buffer solution, the reaction parameters determined from such studies will likely be significant different from interaction of the surface with that protein from whole blood. For example, surfaces covered with chains of poly(ethylene glycol) (PEG) are very effective at resisting the adsorption of proteins from single component

solutions[16], but they are not protein resistant when implanted into the biological environment[31]. Thus, the reductionist approach that works reasonably well for catalytic reactions doesn't work for biological reactions. This means surface analysis methods need to be developed that can be applied directly to the complex biological environment. However, model studies still have a role. They can be used in the development and validation of methods for characterizing interactions of the complex biological environment with the surfaces of biomedical devices[1].

Awareness of the importance of surfaces evolved differently in the catalytic and biological communities. For many decades the catalytic community has been aware of key role surfaces play in catalytic reactions and the importance of doing detailed surface characterization[4]. This appreciation of surfaces developed much later in the biological community[1, 2]. For example, in the 1980s surface analysis methods such as XPS were widely used in catalytic studies. In contrast, during this time only a few researchers were using surface analysis methods in biological studies. In fact, many researchers doing biological studies didn't fully appreciate the role of surfaces or the need for surface characterization in biological studies[1, 2]. This meant an important role of biomedical surface analysis researchers was to educate the biological community about surfaces. While the awareness and appreciation of surfaces in biological studies has significantly improved in the past two decades, there is a continuing need to address this issue. For example, while for decades the catalytic community has widely understood the importance of characterizing the surface structure and composition of nanoparticles used in catalytic applications, it is only in the last ten years that the biological community has realized the need to characterize the surface structure and composition of nanoparticles used in biomedical applications[23].

In analogy to single crystal surfaces used as model catalysts, polymers and self-assembled monolayers (SAMs) have been some of the organic surfaces used as model biomaterials. Many of the first biomedical surface analysis studies focused on polymer surface analysis, using techniques such as high-resolution XPS and secondary ion mass spectrometry (SIMS) to characterize well-defined polymers[32–34]. Like single crystals where the surface chemistry and structure could be systematically varied, the polymer surface chemistry and structure could also be systematically varied. For polymers this involved varying the type of homopolymer (aromatic, acrylate, fluorocarbon, etc.) and changing the composition of a given polymer group (e.g., varying the side chain length), as well as modifying the polymer surface through mechanisms such as chemical derivatization or RF glow discharge deposition. Also, mixed polymer systems, analogous to metallic alloys, can be prepared as either block copolymers or blends of homopolymers, providing another way to systematically vary surface chemistry and structure. While the polymers provide a wide range of surfaces with well-defined chemistries, they were typically disordered. SAMs offered the possibility to prepare surfaces with both well-defined chemistries and structures[35], allowing them to be used as model biomaterials just as single crystal are used as model catalysts. However, there is an important difference between metallic single crystals and organic SAMs. Metallic single crystals can be cleaned and prepared by sputtering with high-energy, monoatomic Ar ions followed by annealing at high temperatures, processes that would completely destroy organic SAMs. In contrast, organic SAMs methods are usually prepared by deposition from solution or vapor. For both metallic single crystals and organic

SAMs it is essential to perform detailed, multi-technique surface characterization after each step involved in their preparation, surface modification and interactions with catalytic or biological environments. Important points for SAM analysis include characterizing the anchor group binding to the substrate[36], the ordering of the chains[37], and the chemistry of the head group[38], as well as any change in these properties that occur when the SAM is exposed to biomolecules and biological environments. Organic SAMs are typically prepared from individual molecules such as alkyl thiols, which can limit their stability (e.g., the Au-S chemisorption bond energy is ~40 kcal/mol). To increase the stability of these organic thin films, polymer backbones with anchor groups on multiple side chain were designed[39]. The multi-point attachment of these films significantly increased their stability and also allowed increased versatility for surface functionalization. However, the advantages of multi-point attachment and increased functionalization capability came with a decrease in the order of the films.

An important set of biomolecules in the biological environment are proteins. These biomolecules can range in size from a few tens of kDa to a few hundreds of kDa. They are typically the first molecules to arrive and interact with the surface of material when that material is placed in the biological environment, so characterizing protein-surface interactions and the structures that result from those interactions is important to understanding how biomaterials interact with the biological environment[40]. However, unlike small chemisorbed molecules where various experimental techniques and theoretical calculations can be used to determine their detailed structure at the atomic level, characterizing the structure of surface bound large proteins is particularly challenging, especially since many proteins are highly flexible and can restructure when they come in contact with a surface. Protein structure can be broken down into primary structural units (i.e., backbone sequence of amino acids), secondary structural units (beta sheets, alpha helices, etc.), tertiary structural units (i.e., how the protein is folded) and sometimes quaternary structural units (e.g., the combination of two or more protein monomers into a complex). Although it is currently not possible to obtain atomic level structure information about large surface bound proteins due to their size and complexity, progress is being made. A complementary approach using both experimental and simulation methods is required[14, 41]. Starting with well-defined, smaller subunits of the larger proteins is a good way to develop the methodology for structure determination of surface bound proteins. Figure 6 shows a 14 amino acid sequence of leucines and lysines that fold into an alpha helix (LK14, MW ~2 kDa), the B1 domain of Protein G (MW ~6 kDa), and albumin (MW ~66 kDa). The first property to determine is the peptide or protein surface coverage. This can be done using methods such as XPS[42, 43], surface plasmon resonance (SPR)[44] and quartz-crystal microbalance with dissipation (QCM-D)[45]. For adsorption from single component protein solutions, these methods can directly determine the surface coverage of that protein. For adsorption from mixed protein solutions, these can only determine the total protein surface coverage. For protein films with more than one protein other techniques such as time-of-flight SIMS (ToF-SIMS) that can provide unique signatures from different proteins are required[46, 47]. ToF-SIMS, when calibrated by ¹²⁵I radiolabeling experiments, has been used to quantify surface coverage of individual proteins in binary and tertiary mixed films[43]. However, when the protein films become more complex, such as those deposited

from blood plasma, only qualitative insights about the composition of the mixed protein film can be obtained[48].

In addition, to determining the coverage and identity of surface bound proteins, it is also necessary to examine their conformation and orientation[49, 50]. For many blood proteins hydrophobic amino acids are predominately located in their inner core while hydrophilic amino acids are predominately located at their outer surface. When this is the case the ratio of hydrophobic to hydrophilic amino acids determined by ToF-SIMS (sampling depth of ~2nm, which is small relative the size of most proteins) can be used to provide insight into protein conformation[51]. However, there are situations where this is not the case (e.g., a hydrophobic amino acid is located on the surface of a protein), so it is always best to examine the actual 3-D structure of protein and determine where specific amino acids are located[16, 52].

Protein orientation can play a significant role in the activity of a surface bound protein. For example, even if a protein retains its native, active structure when bound to a surface, its binding site must be available to bind its target biomolecule (e.g., the F_{ab} region of an antibody must be oriented away from the surface to bind its target antigen). Since PEG based surfaces have been shown to enhance the retention of the native protein structure[16], using PEG chains terminated with head groups that can selectively bind the target biomolecules is a good way to prepare a surface for optimum protein binding activity. An example of the design principles for preparing such a surface is shown in Figure 7. A PEG based disulfide with a maleimide head group on one arm is first assembled onto a Au surface[53]. Then the Protein G B1 domain which has been protein engineered to place a cysteine amino acid either near the C or N terminus is bound to the maleimide head group[54, 55]. This allows the two cysteine mutants to be bound to surface in opposite orientations. The asymmetric distribution of amino acids Protein G B1 shown in Figure 7 can then be probed by ToF-SIMS to characterize the different orientations. Sum frequency generation (SFG) vibrational spectroscopy analysis of the amide I band can also be used to determine the orientations of the beta sheet and alpha helix secondary structures present in Protein G B1, thereby determining the overall protein orientation[14, 55]. Protein G B1 selectively binds the F_c region of immunoglobulin G (IgG), so orienting Protein G B1 with the IgG binding site available is necessary for optimal IgG. It has been shown that the binding IgG is approximately 30x higher for Protein G B1 when the cysteine is located near the N-terminus compared when the cysteine is located near the C-terminus[56].

Techniques such as ToF-SIMS and SFG can provide significant molecular level detail about the structure of surface bound peptides and proteins (e.g., orientation of amino acid side chains in peptides[57] and orientation angles of small proteins[55]), but they can't provide atomic level structural information[41]. For peptides it has been possible to use the molecular level structural information obtained experiment to guide molecular dynamic (MD) simulations, which has produced atomic level structural information about surface bound peptides[58, 59]. The combination of MD simulations with experimental measurements has two benefits. First the MD simulations can extend the experimental results to the atomic level and second the experimental results can be used to validate the force fields, etc. used in the MD simulations. Detailed MD simulations to date has been

limited to peptides and small proteins, but as methods are made more efficient and the speed of computers increase it should be possible in the future to apply MD simulations to larger proteins[60]. In addition, patterned protein surfaces are widely used so obtaining structural information about surface bound proteins in a spatially resolved manner is increasingly important[61]. For all of the analyses using ToF-SIMS, multivariate analysis is a powerful way to exact information about the identity, amount, conformation, orientation and spatial distribution of surface bound protein films[62].

Surface bound proteins have been the focus of this section on biomedical surface analysis, but obviously biomedical surface analysis encompasses a much larger range of activities. These range from detection of surface contaminants on materials to characterizing the structure and composition of biomedical nanoparticles to determining the 3-D distribution of biological species in cells and tissue sections. In addition to the complexity of the biological environment, the biomedical devices placed in that environment can also be complex in terms of the materials they contain (metal, ceramics, polymers, biomolecules, etc.) and their shape (e.g., expanding metal stents), which offer special challenges to the surface analyst.

Summary

A primary objective of surface analysis, whether it involves chemisorption of small molecules onto metallic single crystals, heterogeneous catalysis, biomedical devices or other materials is to obtain a detailed understanding of the surface composition and structure of material involved and correlate those surface properties to the performance of the material. This provides understanding and insights into the material performance as well as identifying important design parameters for developing new and improved materials for the application involved. Recent advances in materials synthesis and surface modifications methods have made it possible to control chemistry at a scale undreamed of only a few years ago. The dimensions of the lateral chemical variations are diminishing, the complexity of the molecules being introduced at the surface is increasing, and the manipulations of the surface moieties are becoming ever more sophisticated. These advances offer great challenges and opportunities for surface analysis to keep pace with materials advances and their application in complex environments. They have also driven the development of improved data analysis strategies for extracting critical information from the large spectral and image data sets obtained from these complex surfaces with modern surface analysis tools.

Acknowledgments

It has been an exciting adventure starting with chemisorption and reactivity studies of small molecules on single crystal surfaces as a graduate student, followed first by industrial catalysis research and then eventually moving to biomedical surface analysis with side ventures into areas such as organic electronics and clean energy. I would like to thank all the wonderful colleagues I have met and interacted with during the course of this journey. I am especially grateful to all my students, post-doctoral fellows, staff and collaborators at the University of Washington for all the enthusiasm they provided and contributions they made to advancing surface analysis. The National Institutes of Health is gratefully acknowledged for support during the preparation of this manuscript as well as supporting the development of biomedical surface analysis at the National ESCA and Surface Analysis Center for Biomedical Problems (grant EB-002027).

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Figure 1.
Schematic Diagram of a UHV System used for LEED, AES and TDS studies of small molecule chemisorption onto single crystal surfaces.

Figure 2. Schematic drawings showing the (111) terrace atoms as well as step atoms for the Rh(331) (top) and Rh(755) single crystal surfaces. A top view of the low-index Rh(111) single crystal surface is shown at the bottom of the figure.

Figure 3.

UHV catalyst treatment system directly connected to an HP 5950A ESCA and used for XPS investigations of supported catalysts. This system contained stations for sample introduction, sample storage, and sample treatment at atmospheric pressure and temperatures up to 600°C in gases such as air, H₂, 1% H₂S/H₂ and various H₂/CO mixtures. The upper right insert shows the chain-driven sample trolley used to transport samples between stations. The lower left insert shows the sample holders used to handle pressed wafers of powdered supported catalysts.

Figure 4. XPS Co 2p spectra from a Co/Al₂O₃ catalyst after sequential calcination, H₂ reduction, 1% H₂S/H₂ sulfidation and recalcination treatments at 480°C for one hour.

Figure 5. Catalyst reaction cell for *operando* XAS experiments at reactions conditions covering wide pressure (UHV to one atmosphere) and temperature (liquid nitrogen to 600°C) ranges. Samples were prepared by pressing self-supporting wafers. Gases that could be used for *operando* experiments included air, H₂, 1% H₂S/H₂ and various H₂/CO mixtures. Heating was done with quartz-halogen lamps. The insert shows the amount of each different Co species varies as the temperature is increased from room temperature to 430°C in the presence of one atmosphere of flowing 1% H₂S/H₂. ○ = Co₃O₄, Δ = CoO, □ = Co, ▽ and ◇ = Co₉S₈.

Figure 6. Structures of a leucine/lysine alpha helical peptide (top), the B1 domain of Protein G (middle) and albumin (bottom).

Figure 7.

Top: Two cysteine mutants of Protein G B1 that bind in opposite orientations to maleimide head groups on PEG chains. Bottom: The assymetric distribution of tyrosine, methionine and leucine/isoleucine amino acids in Protein G B1.

Table 1

XPS determined particle sizes for Co species present on the SiO₂-62 support (surface area of ~300 m²/g and mean pore size of ~15nm) after impregnation, calcination, reduction and sulfidation[18–20].

Technique	Particle Size (nm)			
	Dried Co(NO)_x	Calcined Co₃O₄	Reduced Co	Sulfided Co₉S₈
XPS	~2	18	9	15
TEM		20	8	
XRD		20		