GUIDE TO THE ZARELAB

May 2014 **Department of Chemistry, Stanford**

~Welcome to the ZARELAB~

This booklet has been prepared to make your visit with us more rewarding by presenting a survey of our recent research activities. Each section was written by those members pursuing the work described therein.

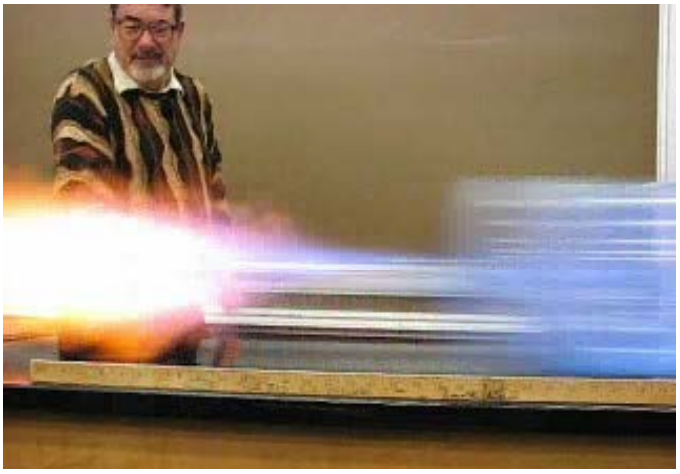
Please feel free to ask the members of my group to discuss any project.

Enjoy your visit!

Richard N. Zare

Stanford University
Department of Chemistry – Mudd Bldg.
333 Campus Drive – Room 133
Stanford, CA 94305
(650) 723-4313

Website:
http://www.stanford.edu/group/Zarelab/



Dick Zare and the rocket test.

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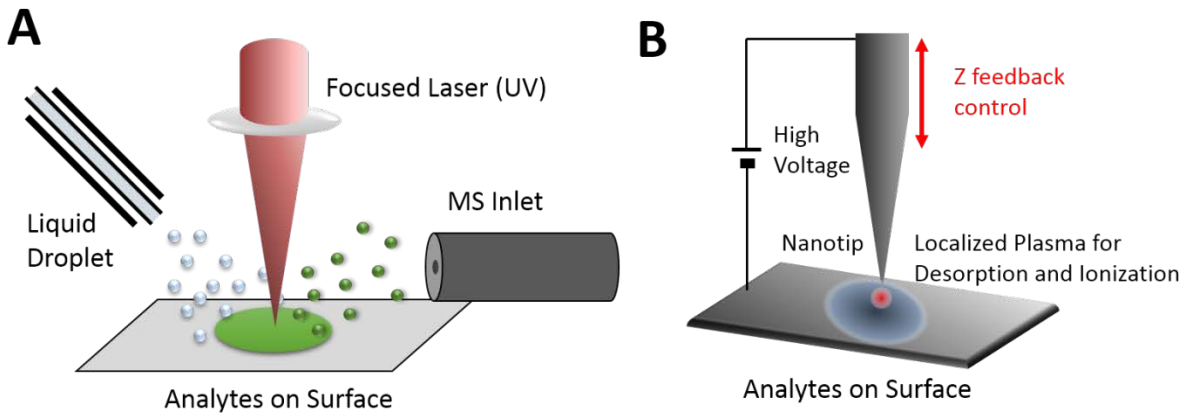

Figure 1: Schematics of (A) desorption/ionization droplet delivery mass spectrometry (LDIDD MS) and nanoambient ionization mass spectrometry (NAIMS)

We are developing new ambient ionization mass spectrometric techniques for high resolution mass spectrometric imaging. We call these new methods desorption/ionization droplet delivery mass spectrometry (LDIDD MS) and nanoambient ionization mass spectrometry (NAIMS). The LDIDD MS utilizes a pulsed laser for desorption and ionization of molecules on a substrate; liquid droplets directly sprayed onto the focused laser irradiation spot carries the desorbed ions to a mass spectrometer. The distribution of different molecules in mouse pancreas tissue, presumably the islets of Langerhans, was successfully imaged. LDIDD MS is also capable of direct real-time analysis of samples in the liquid phase. We are currently seeking the application of LDIDD MS to spatially resolved metabolomic profiling as well as the spatiotemporally resolved secretomic profiling at the single-cell level.

The other ambient mass spectrometry NAIMS utilizes high electric-field-induced plasma at the terminal of a conductive metal tip. The tip of a metal tip is etched to form a sharp nanoscale tip such that a plasma is localized only near the nanoscale tip. A high voltage is applied between the metal tip and metal substrate to generate the localized plasma. The plasma plays roles of both desorption and ionization of molecules on a metal substrate. MS signals from molecules such as caffeine, rhodamine dye, and amino acids as well as MS imaging of a mouse brain slice tissue were successfully acquired. We are applying the NAIMS technique to analyze and image hydrocarbons cracking products on catalytic surfaces.

DESORPTION ELECTROSPRAY IONIZATION MASS SPECTROMETRY IMAGING IN BIOMEDICAL RESEARCH

Livia S. Eberlin

Desorption electrospray ionization mass spectrometry imaging (DESI-MSI) is an ambient ionization technique in mass spectrometry that has recently emerged for imaging biological samples without the need of extensive sample preparation. DESI-MSI has been particularly powerful for investigating the distribution of diagnostic lipids and metabolites directly from tissue sections. Samples are bombarded with microdroplets that dissolve hundreds of lipids and metabolites. The splash forms secondary microdroplets that enter a mass spectrometer, providing a detailed chemical map of the distribution of molecules within the sample surface. Because MSI provides such a wealth of chemical information, this technique invites a statistical analysis.

In the Zarelab, we are using DESI-MSI and biostatistical tools to answer various questions in biomedical research projects. For example, in collaboration with Prof. Dean Felsher, we are answering fundamental questions in cancer research by applying DESI-MSI to investigate the changes in lipid and metabolites profiles that occur in tissue with activation and inactivation of the MYC oncogene in animal models[1,2]. Together with a team of surgeons and pathologists led by Dr. George Poultsides at Stanford Medical School, we are also currently exploring DESI as a tool for gastrointestinal tumor margin assessment[3]. We have been applying the Lasso and the significance analysis of microarrays (SAM) statistical methods in collaboration with Prof. Robert Tibshirani to find molecules that are biomarkers of disease state. In collaboration with Prof. Justin Du Bois, we have shown DESI-MSI potential for investigating the transdermal penetration behavior of various sodium channel modulators[4].

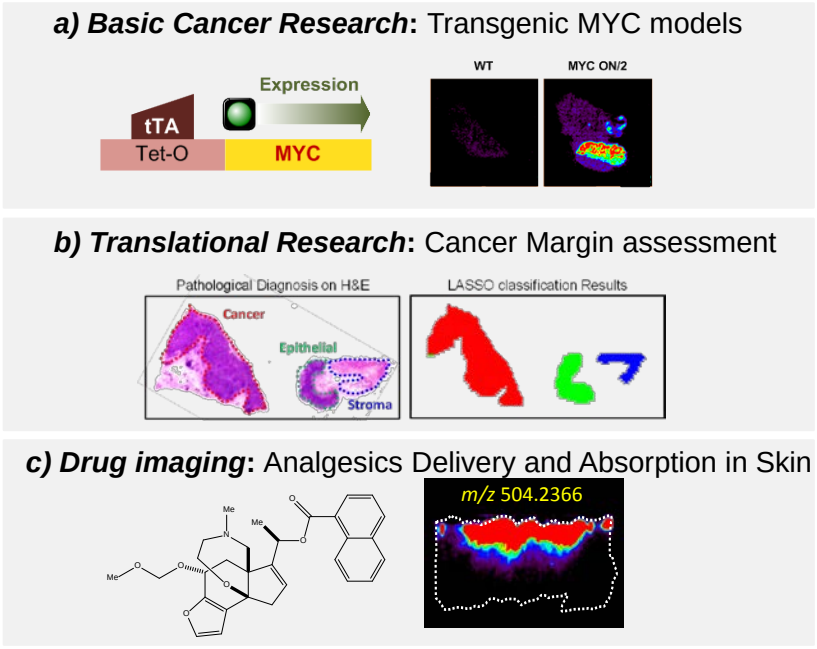

Figure. a) Turning on and off the MYC oncogene to alter the lipid profile; b) use of the lipid profile to asses cancerous from noncancerous tissue during surgery; and c) DESI-MSI of a thin skin slice to determine the extent of penetration of a topically applied sodium channel blocker.

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STATE-TO-STATE REACTION DYNAMICS

Mahima Sneha and Hong Gao

The state-to-state reaction dynamics subgroup in the Zarelab studies gas-phase bimolecular collision dynamics. The three components (v_x, v_y, v_z) of lab frame velocity of the scattering product generated in a bimolecular collision process are measured using a three-dimensional ion imaging apparatus[1]. The lab speed thus obtained is then converted to the differential cross section (DCS) based on the PHOTOLOC method[2] (see Fig. 1). The chemical reaction of hydrogen atom with hydrogen molecule ($H + H_2$) and its isotopic cousins serve as the best prototype for understanding the dynamics of chemical reactions. For this simplest neutral bimolecular reaction, accurate quantum mechanical calculations are now available to compare with experimental measurements. This has taught us much about bimolecular reaction dynamics[3]. Recently we conducted a 30-day rigorous experiment in the hope of observing the geometric phase (GP) effect in the reaction H + HD → HD(v' = 2, j' = 5) + H at a collision energy below 2 eV. Although this collision energy is much lower than the conical intersection (CI) (~2.7 eV) in the H_3 PES[4], the symmetric encirclement of CI by the two interfering pathways, reactive and non-reactive, gives rise to the GP effect, the sole effect of which is to cause a sign change in the interference term. However, this effort has not been successful[4]. An alternative way to observe the GP effect might be to use the F_2 excimer laser (157 nm) to photodissociate HBr or HI to produce fast H atom, which can provide us with collision energies in excess of 3 eV. In this region the GP effect arises from the dynamic encirclement of the CI and is more pronounced according to theoretical predictions. We are currently in the process of optimizing this system.

Another ongoing project is to build up a tunable vacuum ultraviolet (VUV) radiation system using the two-photon resonance-enhanced four-wave mixing method. With tunable VUV, there are several very interesting experiments that can be done for the prototypical $H+H_2$ reaction. The polarization of the rotational angular momentum of the product can be measured using the method of VUV laser induced fluorescence (VUV-LIF). This will provide us with much detailed information about the reaction dynamics. VUV photolysis of HBr and HI can provide us very fast moving H atoms which will have collision energies high enough to energetically access the first excited electronic potential surface of H_3. This will enable us to explore the nonadiabatic dynamics (break-down of Born-Oppenheimer approximation) of this reaction and its isotopic counterparts.

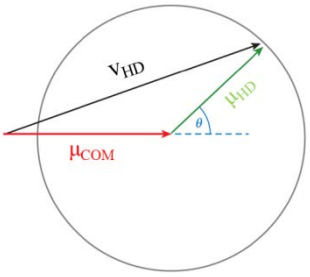

Figure 1: Demonstration of PHOTOLOC method for the reaction $H+D_2 \rightarrow HD(v', j')+H$. μ_{COM} is the center of mass velocity, μ_{HD} is the velocity of HD in the center of mass frame, v_{HD} is the velocity of HD in the lab frame. The scattering angle θ can be obtained by using the Law of Cosine: $\cos\theta = (v_{HD}^2 - \mu_{COM}^2 - \mu_{HD}^2)/(2\mu_{COM}\mu_{HD})$.

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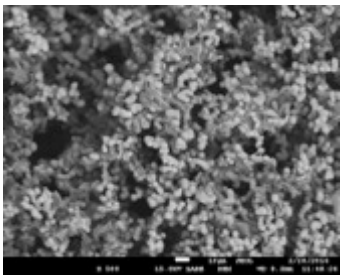
COMPOSITE ORGANIC POLYMERS FOR BIOANALYSIS

Maria T. Dulay and Livia S. Eberlin

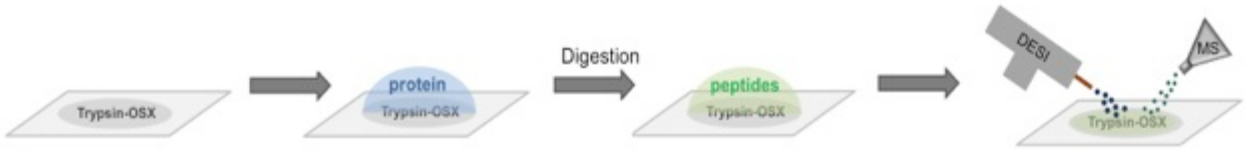
Two of the advantages of using these organic silane-based polymers, called organosiloxane (OSX) polymers, in the analysis of biological mixtures, such as blood or serum, that significantly simplifies analysis are (1) the low reliance on sample preparation protocols and (2) their use with analytical techniques such as mass spectrometry under ambient conditions (i.e., the ionization step is done at room temperature under atmospheric pressure). The ability to directly analyze a complex mixture without the need for sample workup allows for high sample throughput analysis.

Sol-gel chemistry is used to synthesize OSX polymers of high mechanical strength, flexibility, variable porosity and polarity. Bonding or grafting of various chemical entities, such as enzymes, to the surface of these polymers is possible through the active chemical functional groups on the polymer surface. Because these OSX polymers are amenable to various formats, such as planar sheets, bulk porous materials and coatings for various surfaces and materials, they are potentially useful in areas ranging from medicine to drug and environmental monitoring. As such, utilizing the unique properties of these OSX polymers has been a key goal in its development as a platform for rapid detection of analytes, such as drugs and proteins in biological fluids.



Scanning electron microscope image of a porous OSX

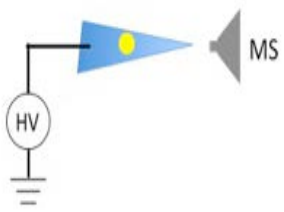
In one platform, biological OSX polymers are being developed as materials for peptide mapping. These polymers have an enzyme, like trypsin, that is grafted by covalent bonding to the surface of the polymer. *In situ* digestion of proteins at room temperature occurs relatively rapidly on the surface of the polymer, which is then followed by direct analysis with desorption electrospray ionization mass spectrometry (DESI-MS) under ambient conditions. High surface coverage of target proteins, including cytochrome *c*, myoglobin and BSA, has been realized without the need for sample preparation and high temperatures, which are quite common in traditional enzyme digestion protocols.



Schematic of *in situ* digestion of protein on OSX-trypsin polymer followed by DESI-MS analysis.

In another platform, porous OSX polymers are used as deposition and spray surfaces for the analysis of drugs in blood and serum. There is no sample preparation. Blood spotted onto the polymer is directly analyzed by ambient ionization mass spectrometry with good signal sensitivity.

Other platforms that involve preconcentration and chromatographic separation using these OSX polymers are being investigated. In summary, this project involves both synthetic as well as analytical endeavors toward the realization of composite organic polymer platforms for rapid analysis of a variety of analytes.



OSX as a spray material for ambient ionization MS.

STRATEGY FOR HIGH-THROUGHPUT SINGLE-CELL GENOMIC SEQUENCING

Yuan Zou and Samuel Kim

In the study of a biological population, how important is individuality? Are the members of the population so similar that the average behavior can describe them all, or are deviations significant enough to make this kind of description misleading? The conventional techniques in biology use a large number of cells and generate the ensemble-averaged values to describe cellular characteristics. These methods are fast and efficient ways of observation as long as the individual cells exhibit little deviation from this average behavior. However, if the deviations are significant, the large-scale ensemble averaging methods fail to give a proper picture of biological phenomena. A simple example will be the case of a bimodal distribution, where the cells with an average behavior actually represent a smaller fraction of the population.

Recent advances in microfluidics opened the possibility of single-cell biology by providing the necessary toolkits for handling and analyzing individual cells. We believe that it is an opportune time to apply microfluidic technologies to investigate individuality of cells because important information relevant to the most pressing biological questions is very likely obfuscated by ensemble averaging techniques. Our section develops techniques for performing single-cell analysis on a microfluidic device. We have made pioneering contributions to the field, including the development of a device capable of capturing a single cell and delivering precise amounts of reagents,[1] an on-chip chemical cytometer integrated with a picoliter micropipette for cell lysis and derivatization,[2] and lysis of a single cyanobacterium for whole genome amplification.[3]

The current goal of our section is to develop microfluidic protocols for amplifying and investigating the genomes of biologically interesting cells at the single-cell level and determining the significance of their genetic diversity. These projects use droplets microfluidic technology to extract and amplify sufficient DNA from a single cell for sequencing (see figure below):

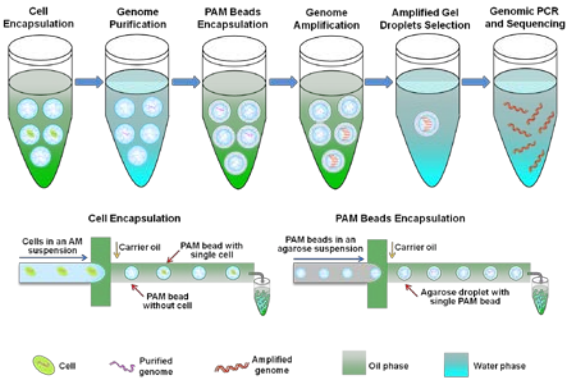


Figure 1: Workflow diagram of strategy for high-throughput single-cell genomic sequencing

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TWO-STEP LASER MASS SPECTROMETRY OF TERRESTRIAL AND EXTRATERRESTRIAL MATERIALS

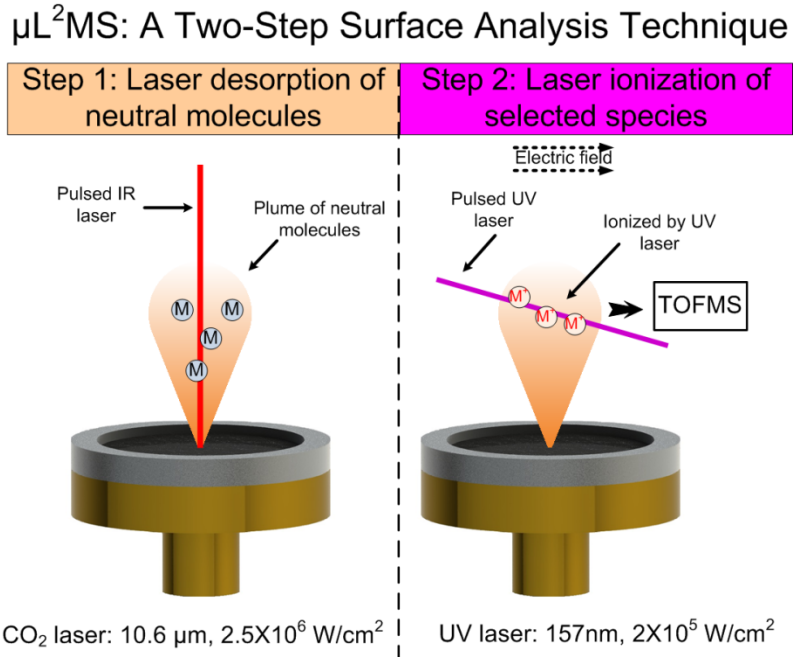
Qinghao Wu

Microprobe laser-desorption laser-ionization mass spectrometry (μL^2MS) is a powerful and versatile microanalytical technique that is used to study organic molecules *in situ* in a wide range of terrestrial and extraterrestrial materials. The combination of focused laser-assisted thermal desorption and ultrasensitive laser ionization provides sensitivity, selectivity, and spatial resolution capabilities that are unmatched by traditional methods of analysis. Over the past decade, this laboratory has developed and applied the μL^2MS technique in a number of different research projects. Some areas that we are currently focusing on are:

Instrument Development: To spread this technology, we are pursuing a way that can reduce the cost and improve the performance. Our plans include: build up a laser desorption ion source using VUV lamp instead of VUV laser to ionize desorbed molecules; build up an ion trap for testing the ion source.

Meteoritics: Analysis of polycyclic aromatic hydrocarbons (PAHs) in meteorites, meteoritic acid residues and interplanetary dust particles.

Petroleomics: This instrument has provided strong proofs for the controversy over the typical molecular structures in asphaltenes, a fraction of heavy oil consisting of highly polar and aromatic molecules. By combined the information of asphaltene from surface-assisted laser desorption/laser ionization mass spectrometry and μL^2MS, we have determined the aggregation number in asphaltene nanoaggregates. Currently, we are studying asphaltenes in various locations. The purpose is to have better understanding on the properties of crude oil.

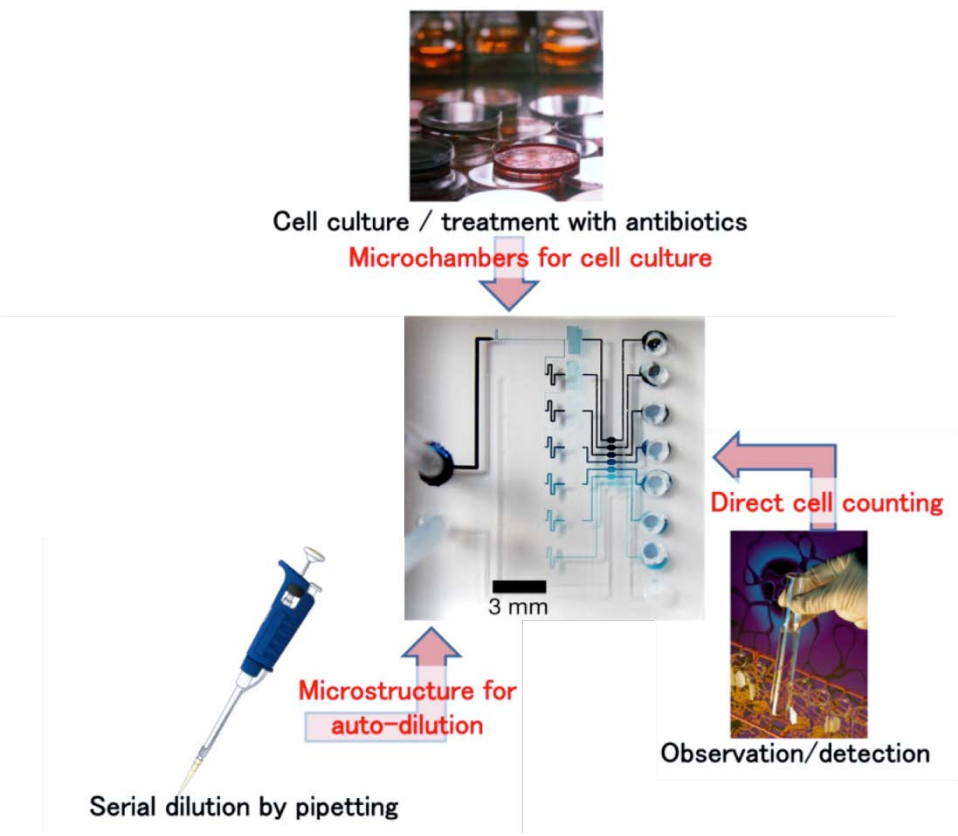


PROTOTYPIC MICROFLUIDIC DEVICE FOR A ANTIMICROBIAL SUSCEPTIBILITY TEST

Stefano Blanco and Samuel Kim

An antimicrobial susceptibility test (AST) is typically performed in clinical microbiology labs to confirm susceptibility of specific bacterial species/isolates to selected antibiotic treatments or to determine drug resistance. The results of the test, in the form of minimal inhibitory concentration (MIC) values or disk diffusion zone diameters, are interpreted and reported to a patient's physician to recommend drug concentrations and combinations for successful eradication of bacteria. The possibility of acquired drug resistance requires constant monitoring of such susceptibility data. Traditional methods require culturing that often takes overnight. Faster AST results can be used to carry out antimicrobial treatment in a more timely fashion.

We are developing a microfluidic device that enables miniaturized AST. Serial dilution of antibiotics concentrations, cell culture, and measurement of cell density will be integrated on a single platform. With this device, the amount of reagents and the duration of testing time can be greatly reduced, thereby lowering the cost per test. Also, the microfluidic platform allows for the possibility of parallel analysis of multiple bacterial isolate samples and/or different antibiotic drugs.



DESIGN OF NANOPARTICLES FOR TARGETED DRUG DELIVERY AND MONITORING THEIR IMPACT ON LIVING TISSUES BY MASS SPECTROMETRY IMAGING

Katy Margulis

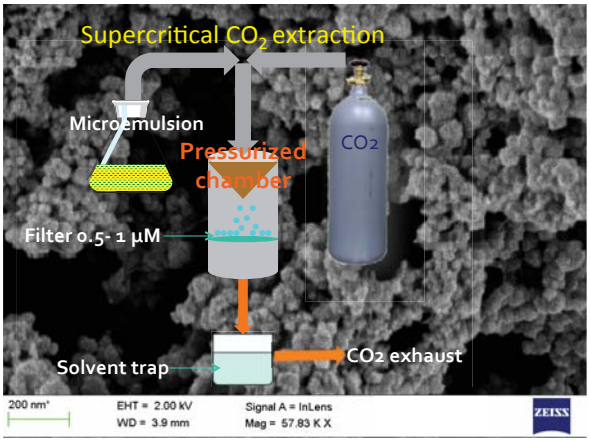


Fig. 1. Schematic representation of nanoparticle formation process with the background of SEM micrograph of the resultant particles

The objective of our study is to engineer organic nanoparticles for efficient delivery of a wide spectrum of therapeutic agents. While the formation of nanoparticles may be achieved by various methods, we put special emphasis on supercritical fluid solvent extraction from volatile microemulsions. This method utilizes nanometric domains spontaneously formed within the microemulsions as templates for nanoparticle synthesis. Desired particle characteristics can be tailored by choosing a suitable initial structure of the microemulsion. Thus, we are able to form nanoparticles of hydrophobic as well as hydrophilic compounds using this method. By drastically decreasing the size of the particles and by employing encapsulating polymers we attain an enhanced bioavailability of active substances.

We utilize carbon dioxide above its critical temperature and pressure (31°C and 73 atm) to extract solvents from the microemulsions. Under these conditions a supercritical fluid is formed. It can act as a potent solvent due to its liquid-like density, thus efficiently extracting solvents from the initial micromulsion system. Yet, as a result of its gas-like molecular diffusivity, the obtained solutions are rapidly removed from the system allowing for particle formation within the confined nanometric domains.

The bioavailability, potency, and target-specificity of the resultant nanoparticles are tested *in vivo* at Stanford Medical School.

During the *in vivo* studies, a spatial distribution of the drug in tissues, as well as endogenous chemical changes elicited by the physiological response to the drug and/or to nanoparticles are monitored by the Desorption Electrospray Ionization Mass Spectrometry Imaging (DESI-MSI). By this mild ionization MS imaging method, tissue sections are scanned directly without any sample preparation and with minimal in-process damage, mapping their chemical composition in two-dimensional manner. To desorb and ionize the target molecules, a beam of charged droplets is directed to the tissue surface, extracting compounds into secondary droplets that are subsequently analyzed by the mass spectrometer. A program-controlled moving stage is used to scan the entire surface of the sample, while the mass spectra are recorded as a function of x,y-position on the tissue. A two-dimensional distribution image with relative signal intensity can be generated for any specific m/z. Hence, a single scan enables acquiring the richest chemical information per pixel, obviating the need for a specific molecular targeting, and allowing for a spatial co-localization of different molecules. Atmospheric pressure ionization conditions, minimal requirements for sample preparation, non-destructive scanning process and high sensitivity make this imaging technique exceptionally valuable for simultaneous tracking of drug localization and chemical changes in tissue caused by pharmacological response.

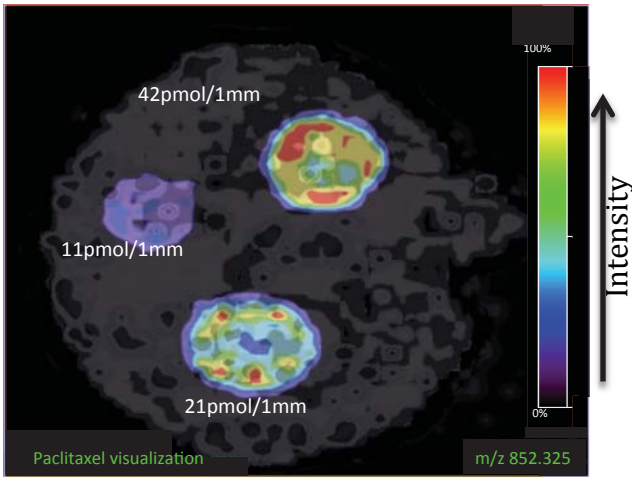


Fig.2 DESI-MSI generated image of anti-tumor drug, paclitaxel, applied in different concentrations on liver tissue (superimposed with the image of the tissue).

NEW DIAGNOSTIC TECHNOLOGY BASED ON CELL-IMPRINTED POLYMERS

Kangning Ren

Cell imprinting is a technology recently developed in the Zare laboratory which produces artificial receptors to cells of interest by template-induced assembly of functional groups on a polymer surface. These cell-imprinted polymers are used to selectively capture the target cells from a mixture. In this method, a prepolymer solution is contacted with a template made of the cells of interest. The prepolymer organizes about the cell template to mimic its shape and in a manner to form the strongest interaction. Subsequently, the polymer is fully cured and the template is removed, leaving imprints in the material which are ready to selectively capture cells of the same species as was on the template. We have proven that the capture mechanism is a combination of recognition of cell shape and chemical recognition of characteristic groups on the surface of the cell.

We are applying this technology to developing diagnostic technology for bacterial infection. Infectious disease is one of the major causes of morbidity and mortality for humans. Quick and accurate diagnosis of infection is the prerequisite to choose proper treatment and to prevent the spreading of the disease. For some infectious diseases, the current diagnostic methods are either too slow or too expensive. We are developing a new diagnostic method based on cell-imprinted polymer film, which will capture the target bacteria in a liquid specimen on the designed spot of a device. With this method, we anticipate the power to selectively concentrate the suspected pathogen from a patient's sample so that detection can be achieved without incubation, thereby greatly reducing the diagnosis time and cost.

Besides patient specimen, the above mentioned strategy could also be applied to detecting the presence of certain microorganism in other samples. One significant application that we are also working on is quick and low-cost on-site food safety test.

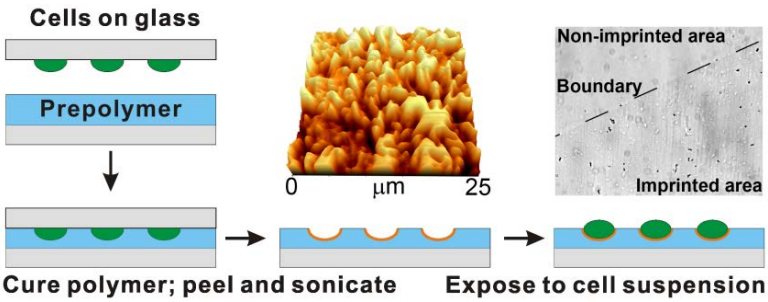


Figure 1: Cell-imprinting of a polymeric film on a microcope slide. Cells are pressed into a prepolymer. Cavities of cell-imprints are left after peel the template and clean the surface. The inset at top centre is an AFM image of polydimethylsiloxane surface imprinted with *M.smegmatis* (*M. smeg*), a surrogate for *M. tuberculosis*. The inset at top right is a microscopic image of the *M. smeg* captured on the imprinted surface. *M. smeg* were stained to be dark.

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ELECTRORESPONSIVE POLYMERS FOR BIOMEDICAL APPLICATIONS

Devleena Samanta

Stimuli-responsive polymers are promising candidates for developing new drug delivery systems. These "smart" materials are attractive as they can potentially provide greater control by releasing drugs only under specific biological conditions, or when an external stimulus is applied.[1] Moreover, their properties can be tailored based on composition, size, shape, structure and morphology. Different kinds of stimuli responsive polymers that respond to pH, enzymes, temperature, irradiation with near IR or UV/VIS light, magnetic field and ultrasound have been investigated so far.[1] This study focuses on the development of a drug delivery system (DDS) that is responsive to electrical stimuli, and aims at understanding the chemical processes that are involved in such a DDS. The advantage of using electric signals is that they are easily generated and controlled, and do not require the use of specialized equipment. Previous reports[2] have shown that biologically relevant molecules can be released from bulk electroactive polymers by applying an electric current or a potential. However, invasive surgery may be required to implant devices based on these systems for successful implementation. In our research, we will employ nanoparticles of these polymers to gain precise control over drug release, increase drug-loading capacity, and facilitate a less invasive method of drug administration.

Pioneering work in our lab has shown nanoparticles of electric field responsive polymers can also be used for drug delivery.[3] Nanoparticles have greater surface to volume ratio, and therefore, greater drug-loading capacity. In addition, particles in the size range 50-100 nm could be circulated and excreted with relative ease. Fluorescein-encapsulated polypyrrole nanoparticles were tested for drug delivery. Here, fluorescein acts as a drug model. The release of fluorescein was monitored *in vitro* as well as *in vivo*. For animal testing, the nanoparticles were loaded into a biocompatible hydrogel, PLGA-PEG-PLGA, which was consequently subcutaneously injected into mice. Preliminary data showed fluorescein is released when a weak potential is applied, and the amount of release scales with the magnitude of the potential. The electric field provides temporal control whereas the hydrogel provides spatial control. The objective of this project is to improve the existing protocol for forming polypyrrole nanoparticles and expand the scope to the incorporation of different drugs.

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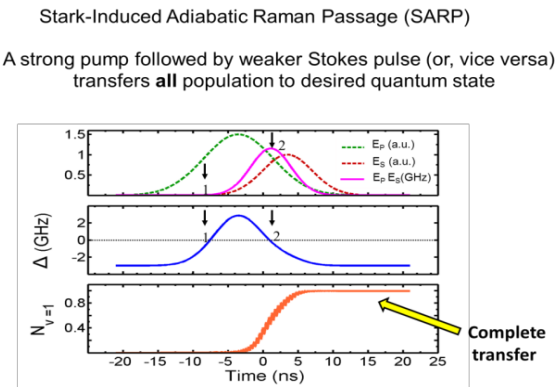
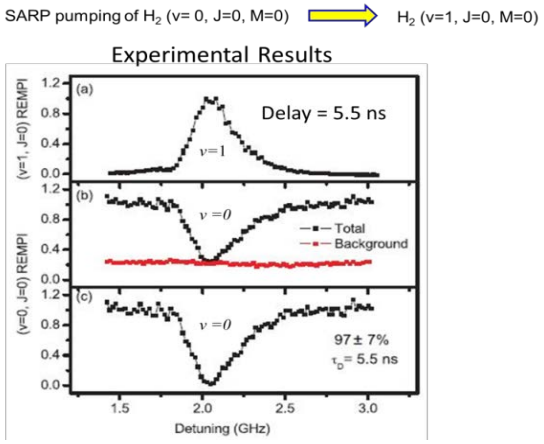
COHERENT PREPARATION OF ROVIBRATIONAL *M*-EIGENSTATE: ITS APPLICATION IN REACTION DYNAMICS

William Edward Perreault, Wenrui Dong, and Nandini Mukherjee

We are studying fundamental chemical reactions such as $D+H_2 \rightarrow HD+H$ at the quantum level by preparing reagent molecules in a single or a coherent superposition of quantum states. To prepare a target molecule in a selected rovibrational *M*-quantum state within the ground electronic surface we introduced a new coherent optical technique called *Stark induced adiabatic Raman passage* (SARP).[1] SARP utilizes light-induced AC-Stark shifts of the molecular energy levels to adiabatically transfer population from an initial to a desired target state by using partially overlapping visible pump and Stokes laser pulses of nanosecond durations (see first figure below). Using SARP we have demonstrated nearly complete transfer of the ground (v=0, J=0) state population of H_2 molecule to a vibrationally excited (v=1, J=0, 2, M) state (see second figure below).[2] This unprecedented control of reagent state will allow us to study stereodynamic processes that previously have been hidden by averaging over the distribution of all M states in unpolarized reagent collisions. Recently we have utilized SARP to prepare a target H_2 molecule in a coherent superposition of M-states within a single rovibrational (v=1, J=2) energy eigenstate[3] thus, preparing $|\psi(t)\rangle = \sum_M C_M |v=1, J=2, M\rangle$ where, the complex coefficients of superposition C_M are controlled by mixing various polarizations of the pump and Stokes laser pulses. In a collision experiment, such coherent superposition of the target state gives rise to interference terms in the differential scattering cross-section, i.e., $d\sigma/d\Omega$ will contain terms proportional to $C_M C_N^*$ representing the off-diagonal density matrix elements for $M \neq N$. Thus, by controlling the relative phase of the coefficients C_M, we can expect to coherently control the outcome of a collision experiment.





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OBSERVING INTERMEDIATES AND DECOMPOSITION PATHWAYS IN C-H FUNCTIONALIZATION REACTIONS USING MASS SPECTROMETRY

Cornelia Flender

The functionalization of unactivated C-H bonds is an important route to synthesize new pharmaceuticals and fine chemicals. In order to develop new catalysts and/or improve reaction yields it is crucial to understand the reaction mechanism. Computational data often provides significant information about the probability of one reaction pathway versus another. However, experimental confirmation is difficult to obtain due to the transient nature of reaction intermediates.

We are collaborating with researchers at institutions across the US to investigate reaction mechanisms and decomposition pathways in transition metal catalyzed C-H functionalization reactions. Mass spectrometry is the method of choice because of its high specificity and mass accuracy. Using desorption electrospray ionization (DESI)[1] coupled to mass spectrometry (MS) (Figure 1), we can access information about the very first milliseconds of a reaction[2]. The reaction is initiated by spraying charged droplets of a reagent onto the catalyst, which is spotted on a surface. Upon impact, secondary microdroplets that contain the reaction partners are released from the surface and directed towards the inlet of a MS. This method offers the exceptional possibility to detect early reaction intermediates, because mass analysis takes place only milliseconds after reaction initiation on the surface.

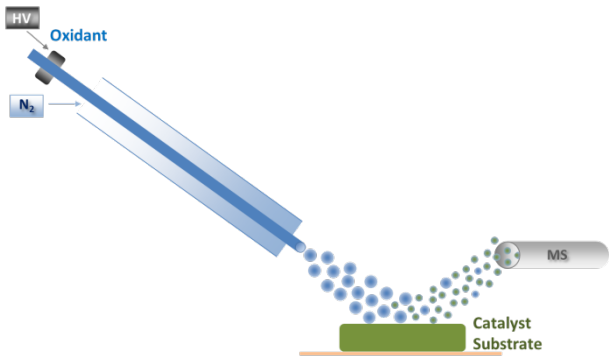

Figure 1: Schematic of the DESI-MS process.

Using this method we have gained insight into the [(Me_3tacn)$RuCl_3$]-catalyzed hydroxylation reaction of tertiary C-H bonds[3]. We found direct evidence of a high-valent dioxo-Ru(VI) species, which is believed to be the active oxidant. Additionally, we found other Ru-oxo intermediates that may also function as competent hydroxylating agents. Studies of incubated reaction mixtures revealed one possible decomposition pathway of the catalyst that proceeds through the formation of a trioxo-bridged Ru-dimer. This information will be used for the development of next-generation catalysts.

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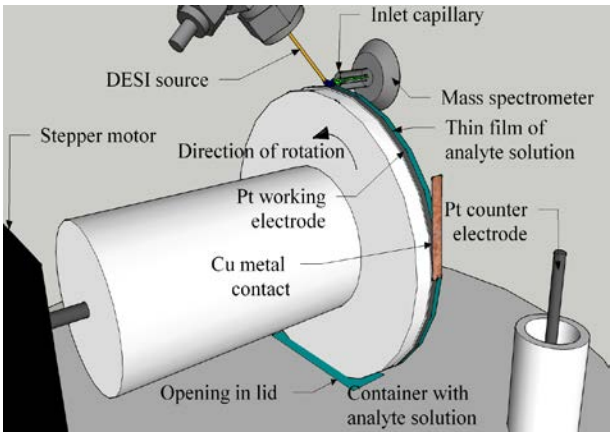
UTILIZING DESPORPTION ELECTROSPRAY IONIZATION MASS SPECTROMETRY TO DETECT ELECTROCHEMICAL REACTION INTERMEDIATES

Tim Brown

Desorption electrospray ionization mass spectrometry (DESI-MS) is a powerful technique for isolating reaction intermediates on the millisecond timescale.[1] We have developed a new means of analyzing electrochemical processes in situ to intercept fleeting intermediates. The working electrode is wrapped around a teflon wheel attached to a stepper motor. The wheel is submerged in analyte solution and the stepper motor rotates the wheel, creating a thin film of analyte solution coating the working electrode surface. A metal contact presses against the working electrode, which is connected to a power supply. The counter electrode is submerged in the analyte solution and also connected to the power supply, completing the circuit.



We sought to isolate reactive intermediates that have yet to be isolated by mass spectrometry. Tryptophan is an amino acid that has been studied electrochemically to try to elucidate the oxidation reactions that take within biological systems.[2] Tryptophan has been studied with cyclic voltammetry[3] and more recently with LC-MS[4], but strong evidence of the first intermediate has yet to be acquired. Using the DESI-ECMS setup we were able to obtain high resolution mass spectrometry and tandem mass spectrometry data supporting the two-electron two-proton oxidation of tryptophan to form the intermediate shown in Scheme 1. The observed mass-to-charge ratio of this intermediate had a direct correlation to when the potential was applied to the working electrode.

Much like tryptophan, uric acid has been studied with electrochemistry and spectroscopic methods,[5,6] however isolation of the direct oxidation intermediate of uric acid has not been accomplished until now. We were also able to use the DESI-ECMS setup to obtain high resolution mass spectrometry and tandem mass spectrometry data for the reactant, intermediate(s), and product of the first steps of electrochemical oxidation, supporting the proposed two-electron two-proton oxidation of uric acid. This is the first high resolution mass spectrometry data acquired for the intermediate species.

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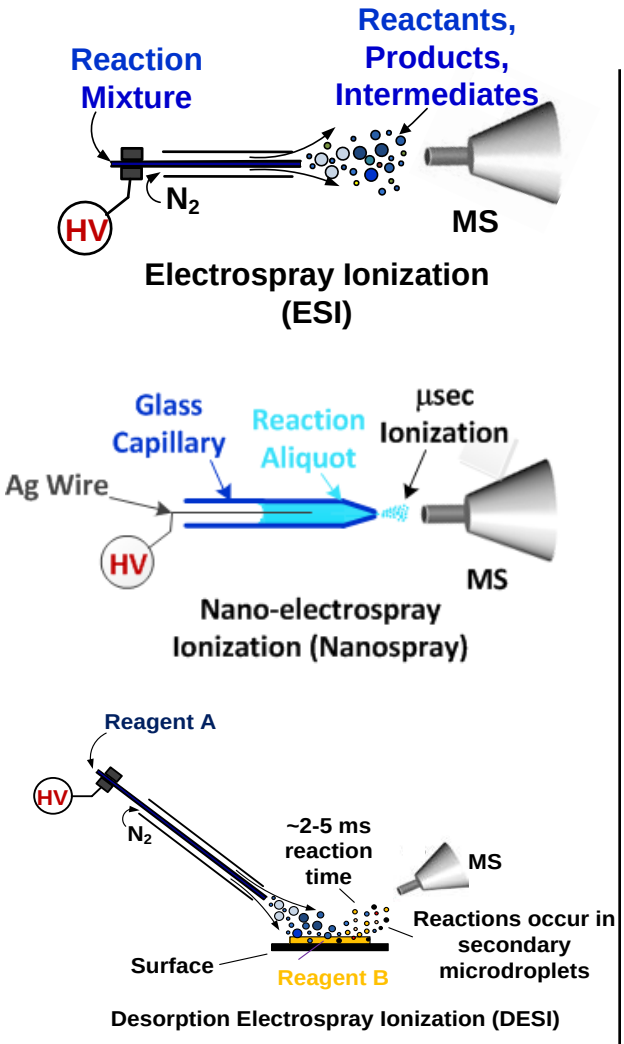
LEVERAGING THE POWER OF MASS SPECTROMETRY FOR MECHANISTIC ORGANOMETALLIC CHEMISTRY

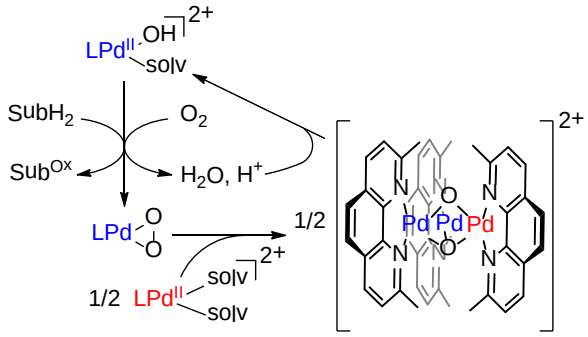
Andrew Ingram

The ability of mass spectrometry to isolate ionic species of interest from complicated reaction mixtures presents a unique opportunity to detect and characterize intermediates in a catalytic cycle. The ion control capabilities of ion trap mass analyzers make it possible to probe the gas-phase structure, spectroscopy, and reactivity of intermediates of important reactions and catalytic cycles.1 Currently in our lab, gas-phase ions are generated directly from reaction mixtures and transferred to a mass spectrometer using a variety of ionization sources including desorption electrospray ionization (DESI), electrospray ionization (ESI), and nano-electrospray ionization (nanospray) (Scheme 1). The unique dynamics of each ionization method enable us to readily study a given reaction from multiple vantage points (e.g. catalyst activation,[1] short-lived intermediates,[2] conventional reaction conditions[3]) and critically assess the information obtained.



Scheme 1: Ionization Methods Employed

By applying these tools to a Pd-based aerobic alcohol oxidation catalyst,[3,4] we have discovered a trinuclear Pd_3O_2 intermediate during catalytic oxidations by Pd. Independent synthesis of this compound, catalysis studies, and isotope labelling indicate that it is a key intermediate in the activation of O_2 by reduced palladium compounds. These studies have provided the foundation for an entirely new mechanism for O_2 reduction by palladium that is relevant to aerobic oxidation catalysis (Scheme 2).



Scheme 2: O_2 Reduction by Pd

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ZARELAB CONTACT LIST

LAB MEMBER			OFFICE PHONE	OFFICE	EMAIL	TITLE
Stefano	**BLANCO**		(650) 723-8280	CLARK E250	scestll@stanford.edu	UG
Tim	**BROWN**		(650) 723-4334	017B	timbrown@stanford.edu	GS
Wenrui	**DONG**		(650) 723-4335	017A	wrdong@stanford.edu	PD
Livia	**EBERLIN**		(650) 723-4334	017B	liviase@stanford.edu	PD
Connie	**FLENDER**		(650) 723-4334	017B	cflender@stanford.edu	PD
Hong	**GAO**		(650) 725-2983	315B	hongao13@stanford.edu	PD
Andrew	**INGRAM**		(650) 723-8029	STAUFFER 1 - ROOM 208	ajingram@stanford.edu	GS
Sam	**KIM**		(650) 723-8280	CLARK E250	slkim@stanford.edu	PD
Jae Kyoo	**LEE**		(650) 723-8280	CLARK E250	jaeklee@stanford.edu	PD
Haifang	**LI**	12/05/13 - 12/05/14	(650) 723-4335	017A	hfli@stanford.edu	VS
Barb	**MARCH**		(650) 723-4313	133	march1@stanford.edu	ADMIN
Katy	**MARGULIS-GOSHEN**		(650) 723-8280	CLARK E250	katym2@stanford.edu	PD
Qianhao	**MIN**	12/04/13 - 12/04/14	(650) 723-4334	017C	qhmin@stanford.edu	VS
Nandini	**MUKHERJEE**		(650) 723-4393	131	nmukherj@stanford.edu	SRA
Willie	**PERREAULT**				perreaul@stanford.edu	GS
Kangning	**REN**		(650) 723-8280	CLARK E250	kangning@stanford.edu	PD
Devleena	**SAMANTA**		(650) 723-8280	CLARK E250	dsamanta@stanford.edu	GS
Mahima	**SNEHA**		(650) 725-2983	315B	mahima@stanford.edu	GS
Qinghao	**WU**		(650) 723-4334	017C	qhwu@stanford.edu	PD
Dick	**ZARE**		(650) 723-3062	133	zare@stanford.edu	PROF
Yuan	**ZOU**	01/07/13 - 12/31/13 - ext. 12/31/14	(650) 723-8280	CLARK E250	yuanzou@stanford.edu	VR

THE ZARELAB


Richard Zare

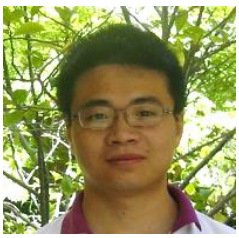

Timothy Brown
Graduate Student


Wenrui Dong
Postdoc

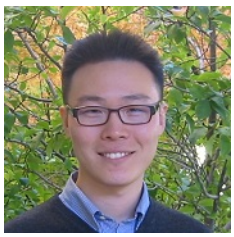

Maria Dulay
Visiting Scholar

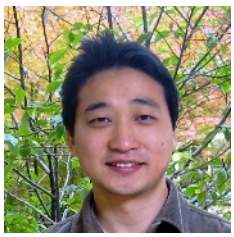

Livia Eberlin
Postdoc


Conny Flender
Postdoc


Hong Gao
Postdoc


Andrew Ingram
Graduate Student


Sam Kim
Postdoc


Jae Kyoo Lee
Postdoc


Haifang Li
Visiting Scholar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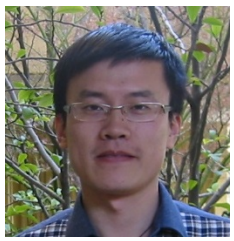
Postdoc


Qianhao Min
Visiting Scholar


Nandini Mukherjee
Senior Research
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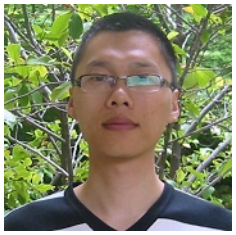
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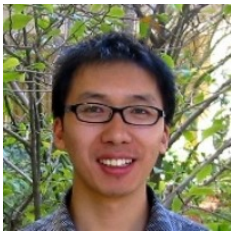
Postdoc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Visiting Scholar

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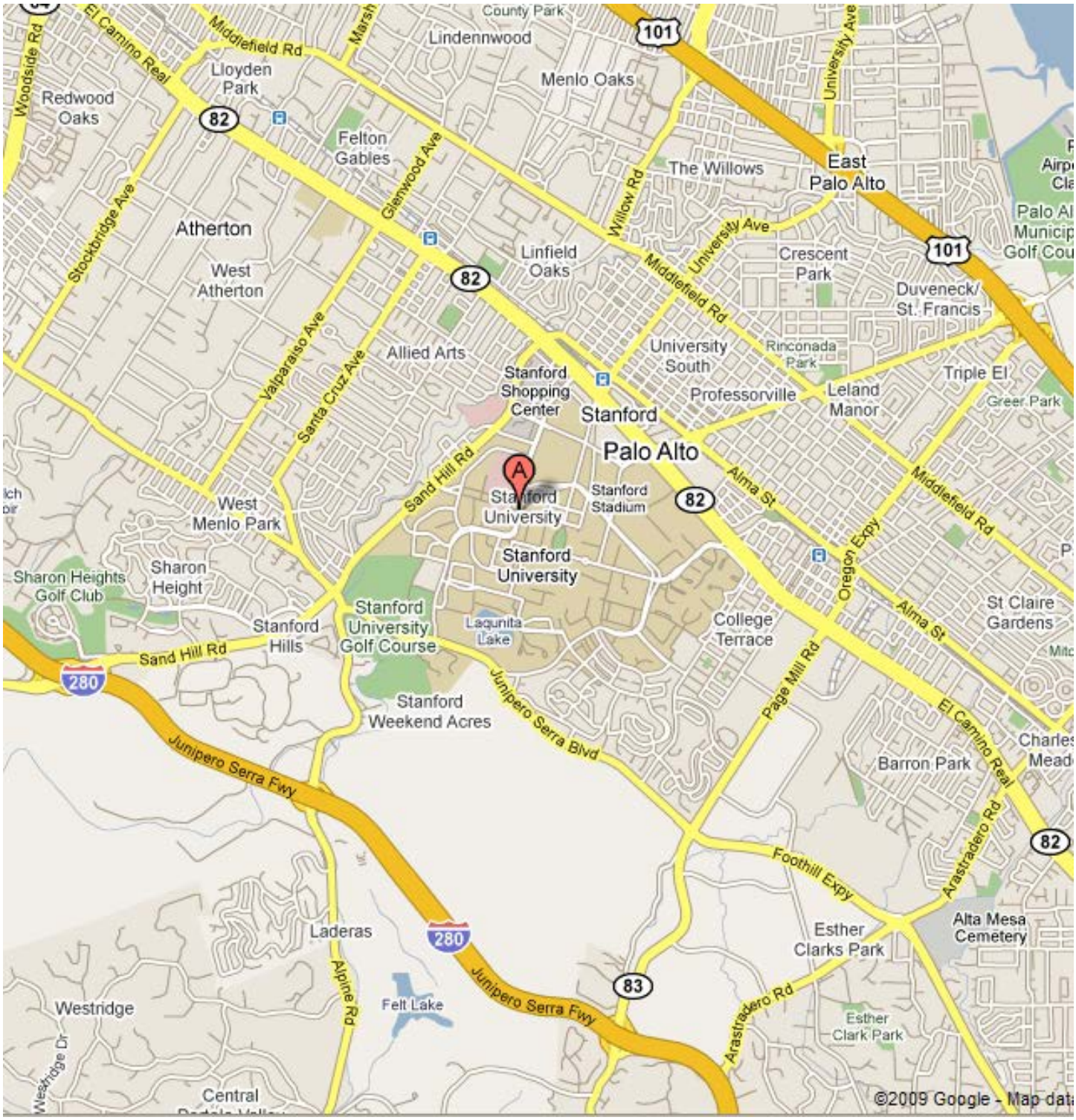

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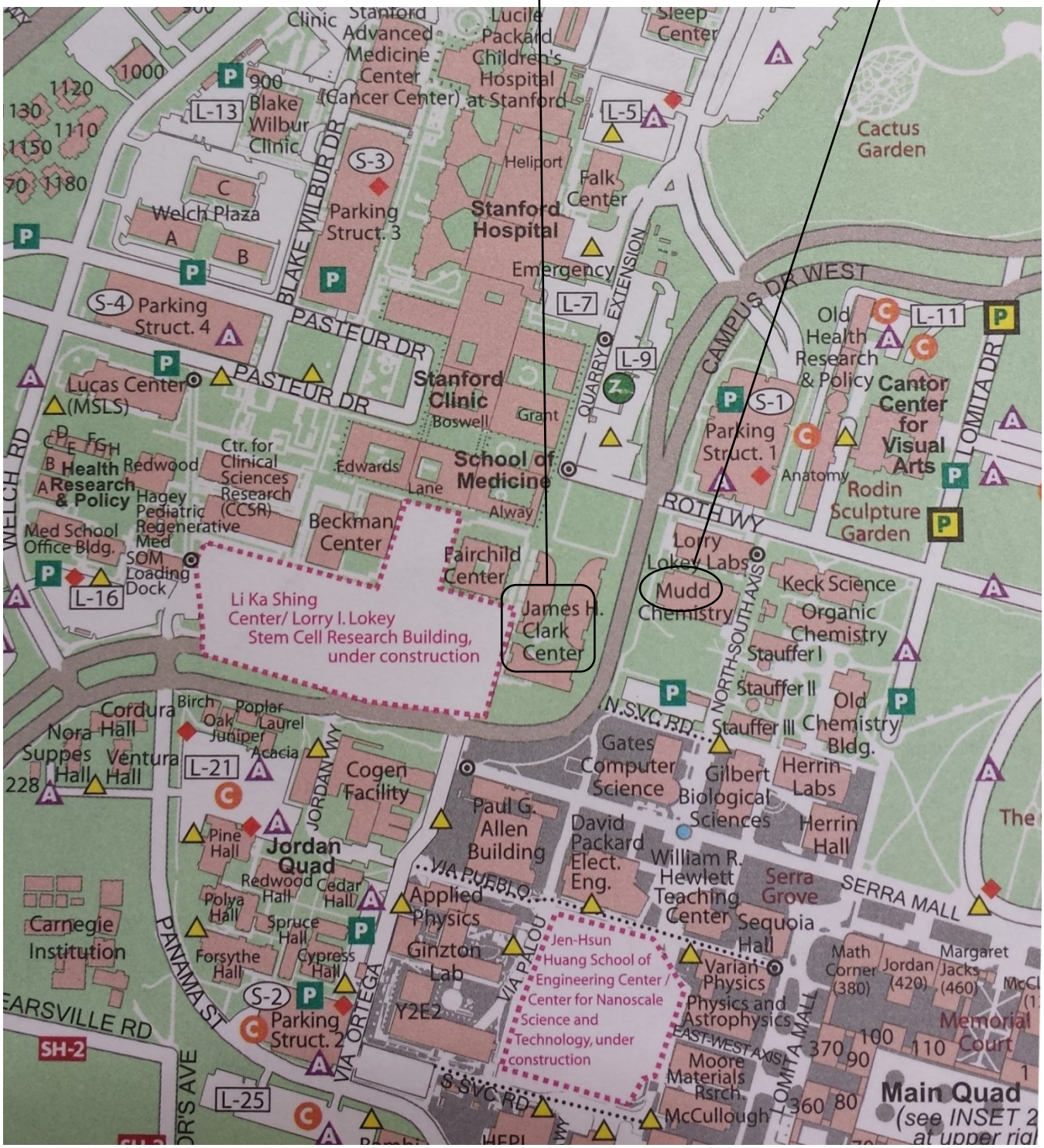

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