Title: Design and application of nanoparticle matrices for matrix-assisted laser desorption ionization mass spectrometry

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Co-PI: Yuping Bao, Assistant Professor, Department of Chemical and Biological Engineering, Adjunct Assistant Professor, Department of Biological Sciences

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A&S Division: Mathematics and Natural Sciences

Carolyn J. Cassady — 10/21/12
Carolyn J. Cassady — Date

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Abstract: The aim of the proposed research is to develop a novel class of nanoparticle matrices for use in chemical analysis employing matrix-assisted laser desorption ionization (MALDI). In MALDI, a laser irradiates a sample/matrix mixture and absorption of photons leads to vaporization and ionization of the sample. Sample ions are then analyzed by time-of-flight mass spectrometry. Molecular mass information can be obtained from large molecules such as proteins, carbohydrates, oligonucleotides, and polymers. The matrix plays a vital role in energy transfer and sample ionization. We have recently discovered that iron oxide nanoparticles (NPs) with various molecules covalently bound to the surface can serve as effective MALDI matrices and have unique advantages over more traditional organic matrices. The "capped" molecules promote energy transfer, while the rigid nanoparticle structure limits the generation of background ions from the matrix. Consequently, these new matrices provide a much cleaner background in the mass spectrum; this facilitates spectral interpretation and allows smaller quantities of sample to be detected. Nanoparticle matrices also led to more extensive in-source decay (ISD) fragmentation of the sample ions. ISD is a technique in which additional energy from the laser causes the sample ions to break apart, which provides information on molecular structure. In addition, as the surface capping of the nanoparticle is modified, these new matrices exhibit selectivity to specific compounds in the sample. The proposed novel class of matrices may lead to new applications for determining the sequences of biomolecules and for imaging the locations of pharmaceutical molecules in tissues.

Proposal: Matrix-assisted laser desorption ionization (MALDI) is a widely used ionization technique for the analysis of molecules by mass spectrometry (MS).[1] The sample is mixed with an excess of matrix compound, which transfers laser energy to the sample, causing desorption and ionization of analytes. Analyte ions enter the mass spectrometer, where molecular mass and structural information are obtained. The choice of matrix is crucial for the success of a MALDI experiment, but selection is often by trial and error. This proposal involves the development of novel new matrices that will broaden the scope of MALDI MS applications.

Iron oxide nanoparticles (NPs)[2,3] with controlled size and shape are being synthesized by the Bao group in UA's Department of Chemical and Biological Engineering. In what is known as a "heat-up" method, the NPs are produced by a simple procedure that is reproducible and gives a narrow size distribution of particles. The resulting NPs intensely absorb UV/visible light, which is an important property for a MALDI matrix because it results in energy transfer from laser photons to the sample. We have recently discovered that iron oxide NPs with different surface capping molecules have unique advantages over traditional organic MALDI matrices. First, NPs provide a cleaner mass spectral background. Attachment of capping molecules to a NP surface reduces matrix molecule self-clustering and fragmentation (a common problem with organic matrices), which in turn minimizes the intensity of low mass background ions that can complicate the mass spectra. Second, NP matrices are versatile and selective because varying the surface capping molecule yields different chemical preferences and targets different analytes in a sample. For example, our preliminary data show that a NP matrix capped with dopamine only ionizes glycans and is transparent to proteins in the test sample. This characteristic is very useful when analyzing target molecules from a mixture. Third, NP matrices allow facile energy transfer to the sample. Due to their ability to absorb and transfer energy from the laser, NP matrices cause abundant fragmentation of sample ions by in-source decay (ISD).[4] ISD is a tandem mass spectrometry (MS/MS) technique in which fragmentation in the MALDI source provides information on

molecular structure. The ability of the proposed NP matrices to promote ISD is an important feature that will increase their utility.

The unique design and properties of our NP matrices should lead to a wide range of applications in the analysis of biomolecules, polymers, and small molecules. We will focus on the fields of glycan (carbohydrate) and protein ISD fragmentation and on MALDI imaging of small drug molecules in tissues.[5] Although several research groups[6-8] have published reports on various NP matrices, the proposed work is novel because no prior research has involved the significant ability of our NP matrices to promote ISD fragmentation and because there has been very little work employing NP matrices for imaging.[9] MALDI imaging detects specific locations of molecules in biological tissue samples. This has importance, for example, in determining how pharmaceutical compounds distribute into human tissue and in locating abnormalities (e.g., cancers) in tissue. MALDI imaging is an emerging area of chemical analysis and our ability to make a major contribution to this field through the development of the NP matrices would enhance the national and international reputations of the Cassady and Bao research groups and of the UA College of Arts and Sciences. This interdisciplinary project is a new research direction for both groups and is also our first collaboration.

The first goal of the project is to demonstrate that energetic NP matrices induce structurally informative ISD fragmentation for glycan and protein samples. Protein glycosylation plays an important role in many physiological processes. Glycan structural analysis by common MS/MS techniques such as collision-induced dissociation and post source decay only leads to relatively uninformative glycosyl bond cleavage. Cross-ring cleavages that reveal important linkage information for sugar units is less routine and requires specialized instrumentation.[10] We have discovered that NP matrices induce extensive cross-ring ISD fragmentation using a standard MALDI/TOF mass spectrometer with a common nitrogen laser.

The example spectrum at the right was obtained using a NP matrix of iron oxide capped with dopamine. This effect will also be tested on N-acetyl and acidic glycans. In addition, protein ISD is an important research area. The new matrices might sequence intact proteins without time-consuming digestion and purification steps. The current limitation of protein ISD is that it often only reveals sequence information near the termini.[4] We will explore the ability of NP matrices to enhance ISD fragmentation efficiency and sequence coverage for proteins.

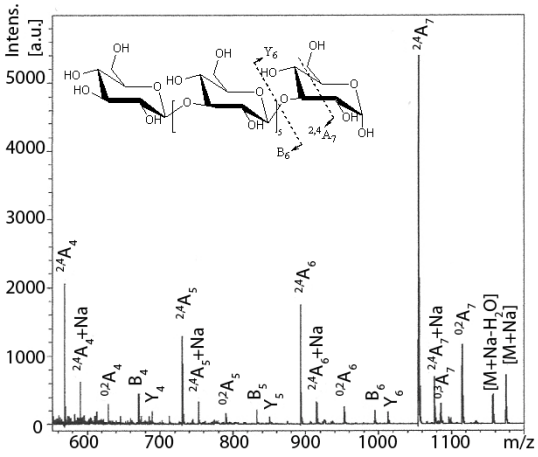

Figure 1. ISD mass spectrum of maltoheptaose (α-1,4) with a NP matrix showing both glycosyl cleavage (B, Y) and cross ring cleavages (A).

The second goal of the project is to demonstrate the selective detection of small drug molecules in cells using NP matrices. Drug and metabolite imaging in tissues has great potential but is currently limited by matrix selection and interferences from matrix ions.[5] NP matrices should overcome these problems; their selectivity and low ion background makes them ideal candidates for imaging. In addition, with ISD fragmentation, both precursor and product ions can be mapped for increased compound identification. Our focus will be on several chemotherapy drugs (e.g., Oxaliplatin and Paclitaxel) to demonstrate the effectiveness and selectivity of NP matrices. Initial experiments will use HepG2 liver cells as model tissue. In our upcoming NIH proposal, funds will be requested to upgrade our MALDI/TOF MS to include imaging capabilities.

We plan to obtain preliminary results that be used in a peer reviewed scientific journal article and in a proposal to the National Institutes of Health (NIH) R01 program. The availability of convincing preliminary data is crucial to obtaining external funding. CARSCA funding would make it monetarily possible for us to obtain preliminary data. The success of this CARSCA proposal will be judged on our ability to obtain external funding and on the number of resulting publications and conference presentations.

Budget:

Reagents for NP matrices synthesis	$2,000
Reagents for cell production	$1,000
Reagents, solvents, and supplies for MALDI analysis	$2,300
User fees for the MALDI/TOF mass spectrometer ($5 per sample)	$1,000
Cost for attending conference to present results	$1,200
Total Requested	$7,500

Budget Justification: The project participants have not been previously funded by CARSCA. Funding is requested for mass spectrometry analyses that will be performed by Dr. Liang and by undergraduate and graduate student members of Dr. Cassady's research group, as well as for the capped NP synthesis that will be performed by student members of Dr. Bao's group. Because of the expense of the chemicals, it would not be possible to perform this work without CARSCA funding. Travel funds are requested to send a graduate student or staff member to the American Society for Mass Spectrometry conference in Minneapolis in June 2013, where the results of this research will be presented. This is the premiere international conference in the field**.**

Timeline: The overall timeline is 1 year. For each candidate NP matrix, synthesis will take several weeks and its MALDI testing will require several days. In a process that could take 3-6 months, synthesis and testing will be performed on a number of candidates before suitable NP matrices for each target compound are found. Then, about 2 months will be needed to perform and refine MALDI experiments with drug-dosed liver cells. The remaining time will be used to test the effectiveness of other NP systems as matrices and to perform any additional experiments that will be included in the peer-reviewed publication and in the NIH grant application.

References:

1. Zenobi R, Knochenmuss R. 1998. Ion Formation in MALDI Mass Spectrometry. Mass Spectrom. Rev. **17**:337– 366.

2. Palchoudhury, S., Xu, Y., Goodwin, J., and Bao, Y. 2011. Synthesis of Iron Oxide Nanoworms. J. Appl. Phy. **109**:07E314 - 07E314-3.

3. Palchoudhury, S., Xu, Y., Goodwin, J., and Bao, Y. 2011. Synthesis of Multiple Platinum-Attached Iron Oxide Nanoparticles. J. Mater. Chem. **21**:3966-3970.

4. Hardoulin, J. 2007. Protein Sequence Information by Matrix-Assisted Laser Desorption/Ionization In-Source Decay Mass Spectrometry. Mass Spectrom. Rev. **26:**672-682.

5. Sugiura, Y., Setou M. 2010. Imaging Mass Spectrometry for Visualization of Drug and Endogenous Metabolite Distribution: Toward In Situ Pharmacometabolomes. J. Neuroimmune Pharmacol. **5**:31-43.

6. McLean, J.A., Stumpo, K.A., and Russell, D.H. 2005. Size-Selected (2-10nm) Gold nanoparticles for Matrix Assisted Laser desorption Ionization of Peptides. J. Am. Chem. Soc. **127**:5304-5305.

7. Tseng, M.C., Obena, R., Lu, Y.W., Lin, P.C., Lin, P.Y., Yen, Y.S., Lin, J.T., Huang, L.D., Lu, K.L., Lai, L.L., Lin, C.C., Chen, Y.J. 2010. Dihydrobenzoic Acid Modified Nanoparticle as a MALDI-TOF MS Matrix for Soft Ionization and Structure Determination of Small Molecules with Diverse Structures. J. Am. Soc. Mass Spectrom. **21:**1930-1939.

8. Wen, X., Dagan, S., and Wysocki, V. H. 2007. Small-Molecule Analysis with Silicon-nanoparticle-Assisted Laser Desorption/Ionization Mass Spectrometry. Anal. Chem. **79**:434-444.

9. Taira, S., Sugiura, Y., Moritake, S., Shimma, S., Ichiyanagi, Y., and Setou, M. 2008. Nanoparticle-Assisted Laser Desorption/Ionization Based Mass Imaging with Cellular Resolution. Anal. Chem. **80**:4761-4766.

10. Asakawa, D., Smargiasso, N., and Pauw. E.D. 2012. Identification and Relative-Quantification of Glycans by Matrix-Assisted Laser Desorption/Ionization In-Source Decay with Hydrogen Abstraction. Anal. Chem. **84**:7463-7468.

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a. Professional Preparation

Pfeiffer College, Misenheimer, NC	Chemistry	A.B., 1979
Purdue University, West Lafayette, IN	Chemistry	Ph.D., 1984
Naval Research Laboratory, Washington, DC	Post-Doctoral Fellow	1988-1989

b. Appointments

Professor of Chemistry, The University of Alabama, 2010-present
Associate Professor of Chemistry, The University of Alabama, 2000-2010
Director of Graduate Recruiting for Chemistry, The University of Alabama, 2004-2008
Associate Professor of Chemistry, Miami University, Oxford, OH, 1996-1999
Assistant Professor of Chemistry, Miami University, Oxford, OH, 1990-1996
Senior Chemist, Midwest Research Institute, Kansas City, MO, 1987-1988
Research Chemist, ARCO Chemical Company, Newtown Square, PA, 1984-1987

c. Selected Publications (of 57 total)

Five Publications Closely Related to This Proposal:

1. "Characterizing the Organic Component of Low-molecular-weight Chromium-binding Substance and Its Binding of Chromium," Y. Chen, H.M. Watson, J. Gao, S.H. Sinha, C.J. Cassady, and J.B. Vincent, *J. Nutrition* **141**, 1225-1232 (2011).
2. "Effects of Transition Metal Ion Coordination on the Collision-induced Dissociation of Polyalanines," H.M. Watson, J.B. Vincent and C.J. Cassady, *J. Mass Spectrom.* **46**, 1099-1107 (2011).
3. "Negative Ion Dissociation of Peptides Containing Hydroxyl Side Chains," D. Pu and C.J. Cassady, *Rapid Commun. Mass Spectrom.* **22**, 91-100 (2008).
4. "A Comparison of Positive and Negation Ion Collision-induced Dissociation for Model Heptapeptides with One Basic Residue," D. Pu, N.L. Clipston, and C.J. Cassady, *J. Mass Spectrom.* **45**, 297-305 (2010).
5. "Negative Ion Production from Peptides and Proteins by Matrix-assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry," J. Gao and C.J. Cassady, *Rapid Commun. Mass Spectrom.* **22**, 4066-4072 (2008).

Five Other Significant Publications:

6. "The Effects of Chromium(III) Coordination on the Dissociation of Acidic Peptides," D. Pu, J.B. Vincent, and C.J. Cassady, *J. Mass Spectrom.* **43**, 773-781 (2008).

7. "C-Terminal amino acid residue loss for deprotonated peptide ions containing glutamic acid, aspartic acid, or serine residues at the C-terminus," Z. Li, T. Yalcin, and C.J. Cassady, *J. Mass Spectrom.* **41**, 939-949 (2006).
8. "Gas-phase acidities of aspartic acid, glutamic acid, and their amino acid amides," Z. Li, M.H. Matus, H.A. Velazquez, D.A. Dixon, and C.J. Cassady, *Int J. Mass Spectrom.* **265**, 213-223 (2007).
9. "Fundamental Thermochemical Properties of Amino Acids: Gas-phase and Aqueous Acidities and Gas-phase Heats of Formation," M.L. Stover, V.E. Jackson, M.H. Matus, C.J. Cassady, and D.A. Dixon, *J. Phys. Chem. B* **116**, 2905-2916 (2012).
10. "A Comparison of the Effects of Amide and Acid Groups at the C-Terminus on the Collision-Induced Dissociation of Deprotonated Peptides," S.S. Bokatzian-Johnson, M.L. Stover, D.A. Dixon, and C.J. Cassady, *J. Am. Soc. Mass Spectrom.* 23, 1544-1557 (2012).

d. Synergistic Activities

1. Chairperson of the American Chemical Society (ACS) Committee that wrote the 2001 standardized analytical chemistry exam (AN-01) from 1998-2001 and a member of the AN-94 committee that wrote the previous exam from 1992-1994.
2. Founder and organizer of the annual Advanced Instrumental Techniques Colloquium (AITC), which annually exposes students from undergraduate schools in Alabama, Mississippi, Tennessee, Georgia, and Louisiana to advanced instrumentation that might not be available at their home institutions, 2002-present. This includes exposure to MALDI/TOF MS.
3. Member of the American Society for Mass Spectrometry since 1981, including secretary and member of the board of directors from 2001-2003, co-founder of the FTMS interest group in 1994, chair of the FTMS interest group from 1995-1997, and member of the Asilomar Mass Spectrometry Conference Committee from 2010-2012 (chair in 2012).
4. On-site reviewer and participant on 4 NSF instrumentation panels, 4 NIH instrumentation panels, 3 NIH study section panels, 1 NIH small business panel, 2 NSF CAREER Panels, and 2 on-site reviews of the Environmental and Molecular Science Laboratory (EMSL) for DOE.
5. Selected as one of the 50 Outstanding Faculty at Miami University, chosen by Phi Gamma Delta service fraternity, 1999. This selection, by undergraduate students, was based on teaching of general chemistry.
6. Past PI or co-PI on 3 NSF grants to obtain funds for the purchase of mass spectrometers, including a MALDI/TOF MS. Past PI on 3 NIH grants, 1 NSF grant, and 1 ONR grant for research involving mass spectrometry (including 1 active NSF grant, which has no overlap with the current project) and co-PI on 1 NIH grant.
7. Taught a graduate level course in mass spectrometry 11 times over the past 20 years.

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(a) Professional Preparation:

University of Washington	Materials Science & Engineering	Ph.D., 2006
Tongji University	Physics	M.S., 1998-2001
East China University of Science & Technology	Chemistry	B.S., 1998
Los Alamos National Laboratory Nanotechnology and Biomaterials, 3/2006-8/2008		Post-doc

(b) Appointments:

Assistant Professor, University of Alabama (2008-current))
Adjunct Faculty of Biological Science, University of Alabama (2009-current))
Postdoctoral Fellow, Center for Integrated Nanotechnologies, Los Alamos National laboratory (2006-2008)
Research Assistant, University of Washington (2001-2006)

(c) Five Publications Closely Related to This Proposal:

1. Palchoudhury, S., Xu, Y., Rushdi, A., Holler, R.A., Bao, Y. "Controlled Synthesis of Iron Oxide Nanoplates and Nanoflowers." *Chem. Comm*. 48, 10499 (**2012**).
2. Xu, Y., Palchoudhury, S., Qin, Y., Macher, T., Bao, Y. "Make conjugation simple: a facile approach to integrated nanostrcutures." *Langmuir*, 28, 8767 (**2012**).
3. Xu, Y., Palchoudhury, S., Qin, Y., Bao, Y. "Water soluble iron oxide nanoparticles with high stability and selective functionality." *Langmuir*, 27, 3966 (**2011)**. *Highlighted in Nature.*
4. Palchoudhury, S., An, W., Xu, Y., Qin, Y., Zhang, Z., Chopra, N., Holler, R. A., Turner, C. H., Bao, Y. "Synthesis and Growth Mechanism of Iron Oxide Nanowhiskers." *Nano Lett.*11, 1141 (**2011**).
5. Palchoudhury, S., Xu, Y., Goodwin, J. Bao, Y. "Synthesis of Multiple Platinum-Attached Iron Oxide Nanoparticles." *J. Mater. Chem*. 21, 3966 (**2011**).

(d) Five other publications of interest

6. Palchoudhury, S., Xu, Y., Goodwin, J. Bao, Y. "Synthesis of Iron Oxide Nanoworms." *J. Appl. Phys.* 109, 07E314 (**2011**).
7. An, W., Wintzinger, L., Turner, C. H., Bao, Y. "A combined Computational/experimental study of fluorescent gold nanocluster complexes." *Nano Life*, 1-2, 133-143 (2010).
8. Keshavarz, Sahar, Xu, Y., Hrdy, S., Lemley, C., Mewes, T., Bao, Y. "Relaxation of polymer coated Fe_3O_4 Magnetic nanoparticles in aqueous solution." *IEEE on Magn.* 46, 1541 (**2010**).
9. Bao, Y., Yeh, H-C., Zhong, C., Ivanov, S., Sharma, J. K., Vu, D. M., Shreve, A. P., Dyer, R. B., Werner, J. H., Martinez, J. S. "Formation and stabilization of fluorescent gold nanoclusters using small molecules." *J. Phys. Chem. C* 114, 15879 (**2010**) .
10. Bao, Y., An, W., Turner, C. H., Krishnan, K. M. "The critical role of surfactants in the growth of cobalt nanoparticles." *Langmuir*, 26, 4780483, (**2010**).

(d) Synergistic Activities:

1. NSF Career Award, 2012
2. Symposium organizer for Materials Research Society, 5/2010
3. Lecturer for the 35th Annual SECME Summer Institute on Nanoscience and Nanotechnology
4. Initiation of Science Party for Kids program with Rock Quarry Elementary School
5. Lecturer for the "Introducing HBCU Faculty to Material Science" Workshop (2009-10)
6. Ralph E. Powe Junior Faculty Enhancement Award, 2010

(e) Prior external funding

1. Title: CAREER: Ultrathin Magnetic Ferrite Nanowires for Bioimaging
PI: Yuping Bao
Agency: NSF
Amount: $493,000
Duration: 8/16/2012-8/15/2017

2. Title: Magnetic-Fluorescent Bifunctional Nanoparticles for Biomedical Applications
PI: Yuping Bao
Agency: NSF
Amount: $226,513
Duration: 8/1/2009-7/31/2013

3. Title: Magnetic Nanoparticle Supported Platinum as Heterogeneous Catalyst
PI: Yuping Bao
Agency: Oak Ridge Associated Universities
Amount: $10,000
Duration: 6/1/2010-5/31/2011

4. Title: MRS Symposium O: Multifunctional Nanoparticle Systems - Coupled Behavior and Applications
PI: Sandra Wolf, **Yuping Bao (Symposium O Chair)**
Agency: US Army Research Office (ARO)
Amount: $5,000
Duration: 4/5/2010-4/9-2010

5. Title: BP/MESC: Experimental Dispersant/Oil/Particulate Formation and Fate (Deep Water) Dauphin Island Sea Laboratory
PIs: Steve Ritchie, Heath Turner, Yuping Bao, Ryan Hartman
Agency: BP
Amount: $13,662
Duration: 1/1/2011-12/30/2011

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a. Professional Preparation

University of Alabama, Tuscaloosa, AL	Chemistry	Ph.D. 2005
Peking University, Beijing, China	Chemistry	M.S. 2000
Peking University, Beijing, China	Chemistry	B.S. 1997

b. Appointments

Mass Spectrometry Facility Manager, University of Alabama, 2005−present

c. Publication closely related to this proposal

1. Rhodes, N. R., Konovalova, T., Liang, Q., Cassady, C. J., Vincent, J. B. 2009. Mass Spectrometric and Spectroscopic Studies of the Nutritional Supplement Chromium(III) Nicotinate. *Biol. Trace Elem. Res.* 130: 114-130.

d. Publications of interest

1. Youn, H.-S., Liang, Q., Cha, J. K., Cai, M., Timkovich, R. 2004. Compound 800, a natural product isolated from genetically engineered *Pseudomonas*: Proposed structure, reactivity, and putative relation to heme d_1. *Biochemistry* 43: 10730-10738.

2. Liang, Q., Simmonds, R. S., Timkovich, R. 2004. NMR evidence for independent domain structures in zoocin A, an antibacterial exoenzyme. *Biochem. Biophys. Res. Commun.* 317**:** 527-530.

3. Chen, Y., Liang, Q., Arciero, D. M., Hooper, A. B., Timkovich, R. 2007. Heme crevice disorder after sixth ligand displacement in the cytochrome c-551 family." *Arch. Biochem. Biophys.* 457: 95-104.

4. Liang, Q., Miller, G. T., Beeghley, C. A., Graf, C. B., Timkovich, R. 2007. Solution conformation of the His-47 to Ala-47 mutant of Pseudomonas stutzeri ZoBell ferrocytochrome c-551. *Biophys. J.* 93: 1700-1706.

e. Synergistic Activities

Member of the American Society for Mass Spectrometry since 2006
Reviewer for 1 NSF instrumentation grant