



Magnetic nanoparticles as mechanical actuators of inner ear hair cells

Dolores Bozovic
UNIVERSITY OF CALIFORNIA LOS ANGELES

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Final Report

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Final report

Grant title: Magnetic nanoparticles as mechanical actuators of inner ear hair cells

Grant number: FA9550-12-1-0407

PI: Dolores Bozovic, University of California Los Angeles

Co-PI: Jinwoo Cheon, Yonsei University

Funding period: 7/15/2012-9/30/2015

Overview of the research program

Hearing constitutes one of the least understood senses, with its remarkable mechanical detection still not fully explained. At the very threshold of audibility, the system is responsive to sub-nanometer motion. At the same time, its dynamic range covers more than six orders of magnitude in pressure. The Bozovic Lab research focuses on exploring the physics behind the extreme sensitivity and robustness of auditory detection.

Hair cells of the inner ear are the biological sensors that detect displacements induced by air-borne or ground-borne vibrations and transduce them into electrical signals that can be sent to the brain. Estimates of the passive mechanical properties of a hair bundle indicate that its thermal fluctuations in water should be almost an order of magnitude higher than the detection threshold. How the auditory system overcomes the effects of noise to achieve its extreme sensitivity is still unknown.

The sensory epithelium is immersed in a fluid environment and sustains prolonged vibrations despite viscous dissipation. In 1948, Thomas Gold first proposed that the auditory system contains an internal energy-consuming amplifier to sustain detection sensitivity under these over-damped conditions. The proposed active process and its role in enhancing the signal-to-noise ratio of detection remains one of the key open problems in this field today.

Traditionally, experiments in this field have used elastic glass fibers or fluid jets to stimulate the hair bundles mechanically. There are a number of studies for which these techniques would be inadequate. The long glass rods introduce significant hydrodynamic drag, and hence the technique cannot be readily generalized to higher-frequency systems. Cheon and Bozovic laboratories therefore established a collaboration to explore the use of magnetic nanoparticles to manipulate hair bundles. Superparamagnetic particles were chemically modified so as to attach to stereocilia of the hair bundle. An electromagnet, positioned in the vicinity of a selected cell by a micromanipulator, was then used to apply a periodic force on the attached particle. We demonstrated that this system could be used to deflect hair bundles at frequencies up to 10 kHz. This technical breakthrough opens new possibilities for generalizing our nonlinear dynamics studies to higher frequency auditory organs.

Year 1: 7/15/2012-7/14/2013

The first year of the grant was focused on achieving three important technical

milestones needed for the project. First, we developed the use of an electromagnetic probe to manipulate the nanoparticles. In our preliminary studies, we used a permanent magnet mounted on a piezoelectric manipulator, which was effective at low frequencies. It allowed us to study effects of offset on hair bundle oscillations, without imposing a load. However, this technique could not be used at high speeds without introducing a hydrodynamic artifact. With the new electromagnet probe, we could readily impose high frequency stimulation.

Second, we introduced the use of smaller particles, 50 nm in size, fabricated by the Cheon group. In our preliminary studies, we used 1 μm sized particles. The small size and more uniform distribution of the nanoparticles minimized the load imposed on a cell while allowing significant actuation.

Third, we have incorporated fluorescence microscopy into our set-up, allowing us to track calcium influx during stimulation. We also developed various labeling protocols for the nanoparticles, allowing us to establish and characterize their attachment to the hair cell stereocilia.

Postdoctoral associate Dr. Michael Levy and graduate student Albert Kao, from the Bozovic laboratory were fully supported by this project. A second graduate student, Seung Ji, was partially involved in the project. Postdoctoral associate Dr. Jae-Hyun Lee and graduate student Seung-hyun Noh from the Cheon group were fully supported by this project.

No publications resulted from the first year of the project, but the main techniques were developed and characterized.

Year 2: 7/15/2013-7/14/2014

In the second year of the grant, we applied our newly developed technique to mechanical actuation of inner ear hair cells. We used magnetic nanoparticles to impose deflections on the hair bundles, mimicking mechanical stimulation that would be imposed by auditory input *in vivo*. Magnetic field provides many advantages over other methods of mechanical manipulation in a cellular system: it readily penetrates biological tissue, is not degraded by light scattering, does not induce heating, and does not require loading with a large probe. The aim was to assess the nonlinear response and phase-locking properties of hair cells with sub-millisecond time resolution. To examine the innate properties of the bundle, it is important to perform this measurement without imposing a mechanical load, and without introducing hydrodynamic artifacts.

We used cubic magnetic nanoparticles, fabricated by the Cheon laboratory, and shown to exhibit enhanced magnetic properties with respect to comparably sized spherical beads. Concanavalin A was shown to exhibit high affinity to the stereocilia and to lead to reliable attachment of particles to hair bundles. Nanoparticles were conjugated with this linker and deposited onto selected areas of the epithelia through a micropipette mounted on a micromanipulator.

Electromagnetic probes were fabricated by winding a fine copper wire around a plastic capillary for ~ 1000 turns and placing them within a water circulation jacket to prevent overheating of the electromagnet. A permalloy 80

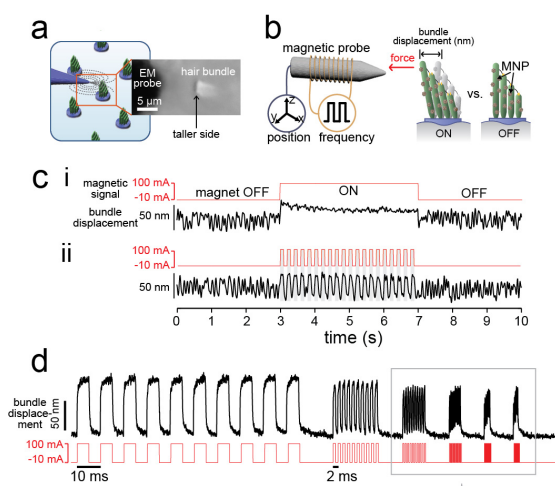


Figure 1. (a) Schematic of a magnetic probe activating a single hair bundle and a light microscope image in top-down view. (b) Schematic diagram of the EM probe stimulating a hair bundle with magnetic particles attached. (c) Motion of a single hair bundle deflected by the magnetic probe. Black line: tracked bundle displacement, red line: current applied to the EM probe. (d) Pulse frequencies of 100, 500, 1000, 2000, 4000, and 5000 Hz were applied to entrain the bundle motion.

rod was used as the electromagnet core material. The magnetic field gradient generated by the probe tip was calibrated by tracking the velocity of magnetic beads (1 μm diameter) immersed in a solution of 80% glycerol. With the magnetic beads in the overdamped regime, the magnetic force is counterbalanced by the viscous force, which can be calculated from the velocity of the particles using the Stokes law. By measuring the trajectories of around 200 magnetic beads, we constructed 2D field gradient maps, thus obtaining a calibration of the magnetic forces produced by various signals sent to the coil. The magnetic probe tip was mounted on a motorized micromanipulator and positioned close to selected hair bundles. The waveform was generated using LabVIEW software to produce step functions of varying durations.

We used this technique to impose mechanical stimulation at frequencies up to 10 kHz (Fig. 1). We showed that the temporal resolution of our system was approximately 1000-fold faster than previously used magnetic switches, allowing us to access the auditory regime of frequencies. The forces exerted by the magnetic manipulation system were sufficient to deflect the hair bundles, whose motion was tracked with high-speed CMOS camera. In addition, we showed that deflections by magnetic particles induced opening of mechanically sensitive transduction channels, by tracking calcium influx into the stereocilia during deflections.

Postdoctoral associate Dr. Michael Levy and graduate student Albert Kao, from the Bozovic laboratory were supported by this project. Two more graduate students, Elizabeth Mills and Tracy Zhang, as well as a visiting undergraduate student, were partially involved in the study. Also, postdoctoral associate Dr. Jae-Hyun Lee and graduate student Ji-wook Kim from the Cheon group were fully supported by this project.

The results obtained during this year were published in the journal ACS Nano:

“Magnetic nanoparticles for ultrafast mechanical control of inner ear hair cells”, J. Lee, J. Kim, M. Levy, A. Kao, S. Noh, D. Bozovic, and J. Cheon, *ACS Nano*, **8**, 6590-6598 (2014).

The findings also received attention in the popular press:

“Nanomagnets could aid study of hearing”, Chemistry and Engineering News, July 22, 2014.

Year 3: 7/15/2014-9/30/2015

In the third year of the project, we focused on using our magnetic nanoparticle technique to study the nonlinear properties of auditory hair cells.

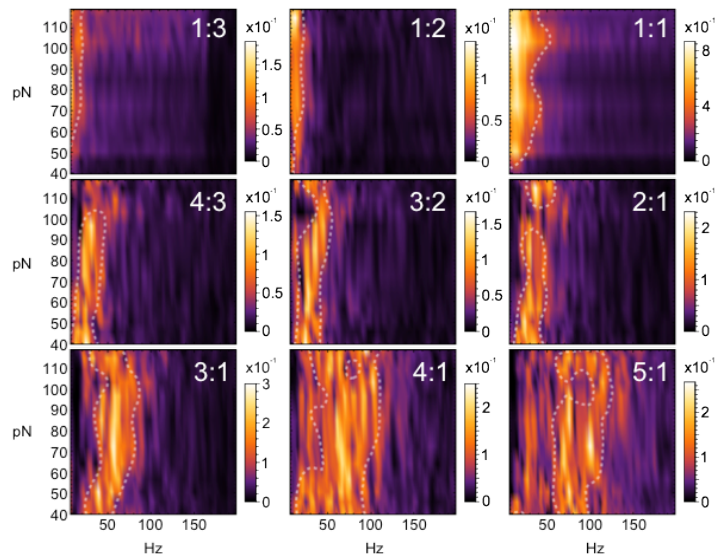


Figure 2. Stimuli are applied at varying frequencies (x-axis) and amplitudes (y-axis). The color code plots the vector strength of the bundle response, at n:m ratios indicated on the panels.

Mechanical gating of the transduction channels in hair cell stereocilia has been shown to lead to a highly nonlinear response of the hair bundle, which is crucial to the sensitivity of detection by the hair cells. Further, it was shown to be ‘essential’ nonlinearity, which is maintained even as incoming signals approach zero amplitude. Most of the studies of this nonlinear response, that have been carried out on single hair cell level, have however been performed at lower frequencies.

readily entrained by even weak stimuli. This spontaneous motility has been shown to be active, requiring an expenditure of energy by the hair cell, to sustain oscillations in a highly dissipative environment. The oscillations are hence one of the signatures of an internal active process in the cell, and have been used to study the nonlinear dynamics underlying the bundle response. The spontaneous oscillations have however only been observed at very low frequencies, below the characteristic range of sensitivity displayed *in vivo*. The question that has remained open in the field is how they might be harnessed to provide amplification of sound, and in particular, if they could lead to detection at faster frequencies.

Under *in vitro* conditions, hair cell bundles can exhibit spontaneous limit cycle oscillations, which are

In a prior study, we showed that the response of hair bundles to stimuli applied across a range of amplitudes and frequencies showed the characteristic triangular shape, known in dynamic systems literature as an Arnold Tongue. The cells showed high sensitivity and sharper tuning at weak amplitudes, and lower sensitivity and no frequency selectivity at higher amplitudes. This is consistent with characteristics of the auditory system *in vivo*.

With our new techniques, we were able to explore higher frequency regimes, stimulating hair bundles of the bullfrog sacculus at frequencies exceeding their natural range. The goal was to observe how phase-locking to a signal occurs in this regime, and at which point it breaks down. In particular, we wanted to test for the presence of higher-order mode-locking, where the natural oscillation of the bundle would synchronize to the imposed drive, but in various $n:m$ ratios of frequencies.

Our measurements showed that the evoked response clearly shows regimes of higher-order mode-locking, which take the form of Arnold Tongues. To determine the degree of synchronization, we calculated the vector strength of the bundle oscillations, at each of the applied stimuli. In Figure 2, one can observe the 2D plots of synchronization, where the color code indicates the vector strength, and the axes denote the stimulus frequency and amplitude. Each of the panels shows a different $n:m$ mode of the entrainment. One of the surprising characteristics apparent in our data is that regimes arise where the hair bundle can phase-lock with different $n:m$ ratios (winding numbers) to the same applied frequency. Under relatively weak stimulation, mode-locking to the imposed signal shows an intermittent flicker between different winding numbers. No phase locking is observed at very weak signals. At intermediate signals, bands appear, indicating phase-locking. At higher amplitudes of stimulation, the bands are sharpened. The overlap between bands indicates that at certain frequencies, the system can phase lock in different ratios to the applied frequency. As the stimulus amplitude is increased, the 1:1 mode-locking is favored.

We proposed in our recent manuscript that the random flicker between the synchronization modes could in fact aid in the detection of higher frequency signals. However, detection would not rely on one hair bundle, but on an ensemble of such hair bundles. As transitions between the modes are random, given an ensemble of oscillators, some fraction of them would exhibit channel-opening or -closing transitions at each cycle of oscillation. We mimicked this scenario by treating sequential recordings from one bundle as equivalent to separate recordings from many oscillators. In Fig. 3, we plot oscillations of a hair bundle subjected to a weak drive; each cycle of oscillation is defined to be between two sequential channel-opening transitions. As shown the plot, the channel closing transition can occur after any number of cycles, but appears preferentially at a particular phase of the stimulus. In an ensemble of such cells, a subset would exhibit this transition at each cycle of the oscillation. Thus, though each individual oscillator is slower than the applied drive, the entire ensemble would reflect applied stimulus frequency and could encode frequencies far higher than those of any of the individual spontaneous oscillators.

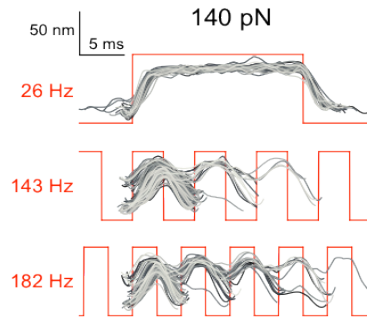


Figure 3. Trains of square waves were sent at varying frequencies, indicated in red. The response of the bundle was cut in segments corresponding to one oscillation cycle, and superposed so that the channel-opening transitions are aligned.

The new technique therefore allowed us to explore a new dynamic regime in hair bundles *in vitro*, and to propose a mechanism by which their innate motility could aid in detection. In future iterations, we hope to apply this technique to auditory systems in other species.

Postdoctoral associate Dr. Michael Levy from the Bozovic laboratory and graduate student Elizabeth Mills were supported by this project for portions of the year. Four more graduate students, Tracy Zhang, Jessica Lin, Justin Faber, and Janaki Sheth were partially involved in the study. Postdoctoral associate Dr. Jae-Hyun Lee and graduate student Ji-wook Kim from the Cheon group were partially involved.

We summarized our findings in the following manuscript, which has been submitted to PNAS:

“Arnold Tongues and high-order synchronization of hair cell bundles”, M. Levy, A. Molzon, J. H. Lee, J. Kim, J. Cheon, and D. Bozovic, *PNAS*, submitted.

The results were also presented at conferences and seminars, including:

Dolores Bozovic (P.I.)

“Nonlinear dynamics and the auditory system”, Seminar, Mechanics and Materials Dept., University of California, San Diego, CA, Jan. 26th, 2015.

“Nonlinear dynamics in the auditory system”, Seminar, International Course on Multiscale Integration of Biological Systems, Institut Marie Curie, Paris, France, Nov. 14th, 2014.

“The physics of hearing”, ‘Evening in the Lab’ Lecture, Center for Biological Physics, UCLA, Feb. 19, 2014.

“Biophysics of inner ear hair cells”, Seminar, NIDCD, Bethesda, MD, Jan. 7, 2014.

“Nonlinear dynamics in the auditory system”, Seminar, UCSB/CWNU/KAIST Workshop on Biologically Inspired Soft Matter, UC Santa Barbara, CA, Sept. 25th, 2013.

“Bifurcations and phase-locking dynamics in hair cells of the inner ear”, Seminar, Nonlinear Dynamics at the Nanoscale, APCTP, Pohang, Korea, Aug. 27th, 2013.

“Bifurcations and phase-locking dynamics in the auditory system”, Seminar, Redwood Center for Theoretical Neuroscience, UC Berkeley, CA, Feb. 20th, 2013.

“Biophysics of hearing”, Seminar, Acoustic Society of America LA Chapter, CA, UCLA, Sept. 18th, 2012.

Jinwoo Choen (Co-P.I.)

“Magnetic nanoparticles: a PRECISION Tool for cell imaging and activations”, Seminar, Nanoparticles at the Interface between Biology and the Materials World, Weizmann Institute, Israel, Jul. 5th, 2015.

“Nanoparticles: A precision platform tool for cell activations”, Seminar, The 4th International Conference on Multifunctional, Hybrid and Nanomaterials, Barcelona, Spain, Mar. 10th, 2015.

“Nanoparticles for cell imaging and activations”, Seminar, 2015 Photonics West, San Francisco, USA, Feb. 8th, 2015.

“Nanoparticles: A Precision Platform Tool for Cell Activations”, Seminar, 2014 ACSB/IFCB Meeting, Philadelphia, USA, Dec. 6th, 2014.

“Nanoparticles for Cell Imaging and Activations”, Seminar, 2014 MRS Fall Meeting & Exhibit, Hynes Convention Center, Boston, USA, Dec. 1st, 2014.

“Magnetic Nanoparticles: A Precision Tool for Cell Imaging and Activations”, Seminar, 5th Frontier Scientist Workshop, KAST, Hotel Novotel London West, London, UK, Oct. 31st, 2014.

“Magnetic Nanoparticles: A Precision Tool for Cell Imaging and Activations”, Seminar, 8th International Symposium on NanoBiotechnology, Beijing, China, Oct. 21st, 2014.

“Nanotools for Biomedical Innovations”, Seminar, 2nd Symposium of the Institute for Basic Science in 2014, Seoul National University, Seoul, Korea, Aug. 5th, 2014.

“Nanoparticles for Cell Imaging and Activations”, Seminar, Korean Medical Association EXPO 2014, COEX Convention & Exhibition 3rd floor, Seoul, Korea, Jun. 28th, 2014.

“Nanotools for Biomedical Innovations”, Seminar, The 5th Yu Ilhan Biomedical Lecture, Seoul, Korea, Apr. 28th, 2014.

“Magnetic Nanoparticles for Cell Imaging and Activations”, Seminar, 2014 Yonsei International Symposium on Nano-Bio Molecular Assembly, Yonsei University, Seoul, Korea, Apr. 18th, 2014.

“Magnetic Nanoparticles for Imaging and Therapeutics”, Seminar, 52nd Annual Autumn Meeting of the Korean Society of Nuclear Medicine, Jeju, Korea, Nov. 16th, 2013.

“Advances of Non-graphene 2D TMC Nanoparticles and Bio-sensing/actuation of Magnetic Nanostructures”, Seminar, The 1492nd Zasshikai Seminar, Chemistry Main Building, The University of Tokyo, Japan, Oct. 4th, 2013.

“Design of Magnetic Nanoparticles for Diagnostics and Cell Activations”, Seminar, China Nano Symposium, China, Sept. 5th, 2013.

“Functional Magnetic Nanoparticles for Diagnostics and Cell Activations”, Seminar, IC ME&D, KAIST Auditorium, KAIST, Korea, May. 15th, 2013.

“Advances of Non-graphene 2D TMC Nanoparticles and Bio-sensing /actuation of Magnetic Nanostructures”, Seminar, AOARD, May. 1st, 2013.

“Functional Magnetic Nanoparticles for Biomedical Studies”, Seminar, Dept. of Chemistry, National Taiwan University, Taipei, Taiwan, Mar. 14th, 2013.

“Functional Magnetic Nanoparticles for Biomedical Studies”, Seminar, Dept. of Chemistry, Cheng Kung University, Tainan City, Taiwan, Mar. 12nd, 2013.

“Functional Magnetic Nanoparticles for Biomedical Studies”, Seminar, NCKU Medical School, Tainan City, Taiwan, Mar. 11st, 2013.

“Magnetic Nanoparticles as Probes and Actuators for Biomedical Sciences”, Seminar, The 9th SPSJ International Polymer Conference(IPC2012), The Society of Polymer Science, Kobe Convention Center, Kobe, Japan, Dec. 11st, 2012.

“Functional Magnetic Nanoparticles”, Seminar, DGIST Global Innovation Festival (DGIF) / Daegu Gyeongbuk Institute of Science & Technology (DGIST), Exco, Daegu, Korea, Dec. 6th, 2012.

“Nanoparticles as Probes and Actuators for Biomedical Sciences”, Seminar, 2012 Dasan Conference-Health Science, Korean Society for Nanomedicine, Gyeongju Hilton Hotel, Gyeongju, Korea, Nov. 13rd, 2012.

“Nanoparticles as Probes and Actuators for Biomedical Sciences”, Seminar, Tateishina Conference, Tateishina Forum, Nagano, Japan, Nov. 10th, 2012.

“Magnetic Nanoparticles as Probes and Actuators for Biomedical Sciences”, Seminar, Kyoto Cell-Material Integration 2012/ Kyoto University’s Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University, Kyoto, Japan, Nov. 8th, 2012.

“Magnetic Nanoparticles as Probes and Actuators for Biomedical Sciences”, Seminar, Inter-Academy Seoul Science Forum 2012, The Korean Academy of Science and Technology (KAST), Westin Chosun Hotel, Seoul, Korea, Nov. 2nd, 2012.

“Rational Design of Nanoparticles for Biomedical and Energy Applications”, Seminar, KIST Materials Forum, Korea Institute of Science and Technology Korea Institute of Science and Technology, Seoul, Korea, Oct. 31st, 2012.

Michael Levy (Post-doc, Bozovic group)

“Nanomagnetism : a New Tool to Probe Auditory Hair Cells”, Oral presentation, Gordon Research Conference, Lewiston, ME, Jul. 2014.

“Fast and Local Mechanotransduction Control via Magnetic Nanoparticles: Mechanical Stimulation of Auditory Cells”, Poster, Biophysical Society Meeting, San Francisco, CA, Feb. 2014.

Elizabeth Mills (Graduate student, Bozovic group)

“Probing hair cell mechanics with magnetic nano-particles: suppression and recovery of limit cycle oscillations”, Poster, Biophysical Society Meeting, Baltimore, MD, Feb. 2015.

Ji-wook Kim (Graduate student, Cheon group)

“Ultrafast Mechanical Control of Inner Ear Hair Cells Using Magnetic Nanoparticles”, Poster, Pioneer Nanoseoul Forum, Yonsei University, Seoul, Korea, Oct. 2014.

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Dolores Bozovic

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Reporting Period Start Date

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Abstract

The collaborative project was designed to develop the use of magnetic nanoparticles to manipulate auditory hair cells. Superparamagnetic particles were chemically modified so as to attach to stereocilia of the hair bundle. An electromagnet, positioned in the vicinity of a selected cell by a micromanipulator, was then used to apply a periodic force on the attached particle. Magnetic field provides many advantages over other methods of mechanical manipulation in a cellular system: it readily penetrates biological tissue, is not degraded by light scattering, does not induce heating, and does not require loading with a large probe. The aim was to assess the nonlinear response and phase-locking properties of hair cells with sub-millisecond time resolution. To examine the innate properties of the bundle, it is important to perform this measurement without imposing a mechanical load, and without introducing hydrodynamic artifacts.

We demonstrated that this system could be used to deflect hair bundles at frequencies up to 10 kHz. This technical breakthrough opens new possibilities for generalizing our nonlinear dynamics studies to higher frequencies auditory. These findings were published in ACS Nano, and the technical innovation was further reported in Chemistry and Engineering News. The technique was applied to explore higher frequency regimes, stimulating hair bundles of the bullfrog sacculus at frequencies exceeding their natural range. The goal was to observe how phase-locking to a signal occurs in this regime, and at which point it breaks down.

Our measurements showed that the evoked response clearly shows regimes of higher-order synchronization. Our data showed that regimes arise where the hair bundle can phase-lock with different n:m ratios to the same applied frequency, with an intermittent flicker between different winding numbers. The results provided a potential model for how innate active bundle motility could be harnessed to detect higher frequency signals.

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Change in AFOSR Program Manager, if any:

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Extensions granted or milestones slipped, if any:

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Reporting Period

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Long & Lat:	Lat: 34.07080078125, Long:-118.37619781494