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RPPR Final Report
as of 16-Sep-2021

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Proposal Number: 69599MS **Agreement Number: W911NF-17-1-0141**
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Major Goals: Solution-phase self-assembly of nanoparticles into mesoscale structures via engineered nucleic acid linkers is a promising strategy for constructing functional materials and dynamically adaptive materials architectures from nanoscale components. Access to such systems is critical for realizing a wide range of defense-related capabilities including ultrasensitive surface-enhanced Raman scattering (SERS) detection of chemical or biological agents, manipulation of the electromagnetic spectrum and photonic cloaking, development of autonomous inorganic ‘organisms’, or novel approaches to quantum information processing.
The microscopic processes governing DNA-mediated self-assembly and reconfiguration remain poorly understood. Conventional experimental approaches can assess only the final assembly outside the native solution environment or follow the degree of ordering but do not provide access to real-space pathways and dynamics in the native solution environment, especially at small scale. Nanometer-resolution imaging approaches have large potential to advance our understanding of DNA-guided nanoparticle assemblies, and can support the exploration and harnessing of their technological potential. Here we use real-time observations by in-situ liquid cell electron microscopy (LCEM) and complementary in-situ imaging approaches, coupled with numerical simulations in a research program aimed at establishing the fundamental mechanisms, pathways, and forces that govern DNA-guided nanoparticle self-assembly and dynamic reconfiguration in the native liquid environment. Our approach provides (i) mechanistic insight at the level of single particles into nucleation, growth, fluctuations, melting, and dynamic reconfiguration; and (ii) quantitative data on key parameters, such as the nucleus size, or interparticle spacing and coordination in small clusters, which can be used as input for computational models to determine the interactions that drive and stabilize assembly in different environments. To actively influence self-assembly and reconfiguration during imaging, we develop and deploy novel experimental platforms comprising liquid cells with tight temperature control and complex fluidic capabilities for controlled release and mixing of solutions. Finally, real-time imaging experiments will be used to investigate biomimetic mineralization within soft assemblies to develop fixation pathways that will enable their use in dry environments.
Specific goals of this research program are briefly summarized below:
A: DNA-guided nanoparticle self-assembly
Our in-situ imaging experiments focus on systems that have shown robust self-assembly, specifically Au particles conjugated with single-stranded DNA and interacting via hybridized linkers with well-defined ‘sticky end’ recognition regions. Goals include:
A.i) Sample preparation: Development of a robust in-house capability for preparing DNA-Au nanoparticle conjugates.
A.ii) In-situ imaging of DNA-directed nanoparticle superlattices: Development and validation of robust in-situ imaging methodologies for DNA-directed nanoparticle superlattices based on LCEM and exploration of

complementary in-situ imaging approaches for such systems.
A.iii) Suppression of radiation damage: Establishing the stability and possible damage pathways of DNA-nanoparticle conjugates in solution under electron irradiation; identification of low dose imaging techniques suitable for extended observation of intact DNA-nanoparticle conjugates.
Microscopy in liquids is used to understand the different stages of the assembly process, with focus on those that involve monomers, small clusters, or interactions between mesoscopic crystals and are thus hard to pinpoint in scattering experiments:
A.iv) Nucleation and growth of the crystalline phase: Nucleation pathway, critical nucleus; fluctuations; nucleation density, recrystallization.
A.v) Shape/size control and coarsening of supracrystals: Shape evolution of growing mesocrystals; attachment kinetics; solution and on-surface mass transport; coarsening.
A.vi) Tunable crystallization and melting pathways: Effects of interaction strength, (tuned via linker density, recognition sequence, displacement strands); pre-melting and melting pathways.
A.vii) Programmable aperiodic structures: Generation of defects and disorder; transition to jamming; bond length and -angle distributions; preferred short- and medium-range motifs.
A.viii) Novel phase behavior: Thermal fluctuations within a widened solid-liquid coexistence window; assembly along reentrant liquid-solid-liquid transitions; temperature-programmed transitions between crystalline phases.
Key questions have only been accessible via calculations, which need to be validated by experiment to ensure that the underlying interactions are properly represented and the predicted structures kinetically accessible. Here, microscopy and complementary calculations are carried out hand in hand:
A.ix) Computer simulations: Development of computations that complement the in-situ experiments and provide insight into the driving forces and pathways for assembly.
B: DNA-based real-time reconfigurable assemblies
The study of dynamic reconfiguration processes benefits from the development of novel experimental capabilities, e.g., the controlled manipulation and mixing of fluids in the observation region of the liquid cells:
B.i) Platforms for fluid delivery and mixing: Development of experimental platforms that can introduce solvent modifiers and reprogramming DNA during microscopy.
B.ii) Alternative imaging methods: Exploration of alternative approaches for nanoscale in-situ imaging in solution.
Our ultimate goal is to understand the physics of DNA-guided assembly and enable the realization of functional materials whose properties can be adjusted in real time under control of stimuli-driven feedback loops. Two key issues exist: Slow reaction rates and reversibility. Practical applications require the rapid response of DNA-nanoparticle matter to applied external stimuli, but most demonstrations to date have shown slow kinetics. A second important issue is reversibility, i.e., the ability to move on demand between endpoint structures in a narrow temperature window in which the crystals are stable. We address reconfiguration processes, rates, reversibility, and the effects of possible unknown boundary conditions with a series of individual goals in mind:
B.iii) Elementary processes and reconfiguration rates – Solvent and solute effects: Lattice parameter tuning by changing ionic strength or pH; addition of co-solvents; effects due to solutes that affect osmotic pressure, intercalate in DNA, etc. Diffusion processes; phase nucleation; progression of volume changes; possible reaction bottlenecks and boundary conditions affecting the reconfiguration; reversibility.
B.iv) Elementary processes and reconfiguration rates – Reprogramming of DNA linkages: Dynamic exchange of linkers, and addition of reprogramming strands; response to changes in DNA linkages; continuous changes vs. phase nucleation; reaction kinetics; progression of reaction fronts; mass transport and crystal relaxation pathways; reaction bottlenecks and jamming.
C: Fixation of DNA-mediated nanocrystal assemblies
Whereas this program focuses on a fundamental understanding of DNA-guided self-assembly toward novel materials and adaptive architectures that perform their function in solution, a minor effort is aimed at exploring novel approaches for fixing DNA-guided assemblies for use in dry environments. Specifically, we study biomimetic mineralization in an electrically insulating calcium carbonate matrix. By introducing Ca^{2+} and $(CO_3)^{2-}$ ions in the solution we address the mechanisms leading to the formation of $Ca(CO_3)$ in the space between the assembled nanocrystals and establish if such approaches may be viable as advanced fixation strategies.

Accomplishments: 1. Development of robust in-house DNA conjugation protocols (Goal A.i) [Refs. (6) & (8)]: We built the laboratory infrastructure and developed reliable protocols for conjugating nanoparticles and other nanostructures with DNA, and for inducing self-assembly by adding linker DNA followed by heating (‘melting’) and slow cooling (‘crystallization’). Using these protocols we successfully crystallized superlattices of different-size Au particles, anisotropic crystals, nanocubes, hollow shells, etc. (Fig. 1) using different combinations of particle-bound and linker DNA. Fig. 2 summarizes our standard DNA oligo designs, which allow tuning key parameters such as DNA ‘bond’ length/strength, particle size, etc., and affect self-assembly and reconfiguration. Development of robust protocols led to assemblies with new functionalities. For instance, we demonstrated for the first time DNA-

conjugation (Fig. 3) and self-assembly of hollow nanocages into programmable superstructures (Fig. 4) in aqueous solution. CL spectroscopy demonstrates a collective behavior with strong global localized surface plasmon resonance enhancement at the surface of the supracrystals, i.e., promising functionality for plasmonics (Figs. 5, 6).
2. Stability of DNA-nanoparticle conjugates and assemblies under electron irradiation (Goal A.iii) [Ref. (1)]: Adequate stability of DNA-nanoparticle conjugates and their assemblies under the electron beam is key for real time observations using liquid cell electron microscopy (LCEM). We devoted significant effort to the identification of possible degradation pathways and developed low-dose imaging techniques that allow extended observations without damaging DNA. We established the radiation damage mechanisms of DNA-nanoparticle assemblies (Fig. 7) and carried out systematic experiments with different linkages to identify the radiation damage pathways (Fig. 8). This led to the following picture of the damage progression in solution: An initial pairwise approach to the spacing of 'dummy'-linked assemblies is consistent with a dissociation of the recognition region followed by complete linker detachment, likely due to radicals produced by the electron beam. A progressive further reduction in particle spacing indicates DNA strand breakage. Based on these findings, we identified low-dose imaging conditions that allow long-time in-situ LCEM of DNA-nanoparticle superlattices by suppressing radical generation and radiation damage.
3. Self-assembly of DNA-nanoparticle and nanorod conjugates into mesoscale structures (Goals A.iv – A.vi, B.iii) [Refs. (2), (7) & (8)]: Efforts to develop alternatives to conventional base-pairing interactions for building DNA-directed superlattices demonstrated self-assembly triggered by a lowering of the solution pH for Au particles conjugated with single-strand (ss) DNA without the need for linkers (Fig. 9). Transient pH lowering in LCEM enabled following the ssDNA-nanoparticle conjugates in-situ and identifying their self-assembly processes. Fig. 9 illustrates this for 17 nm Au nanoparticles conjugated with ssDNA. Real-time observations showed that monomers and dimers are mobile in solution, established the nucleation and growth mechanism of ordered assemblies, and identified characteristics such as preferred structural motifs, interparticle separations, stable nuclei, etc. in the native solution. Assembly via incorporation of monomers, dimers, and larger multimers results in the formation of ordered crystallites. The interparticle spacing in solution is consistent with interdigitation of poly-A duplexes on neighboring particles. Nanoparticle tracking confirmed modifications to the DNA conformation at low pH associated with the protonation of adenine bases and poly-A duplex formation, which governs the interaction between ssDNA-Au nanoparticle conjugates.
We further established ss-DNA driven self-assembly as a general approach applicable also to anisotropic nanostructures conjugated with ssDNA. Two types of Au nanorods – short (aspect ratio: 2.4) and long (aspect ratio: 5.6) were functionalized with ssDNA. As for ssDNA-Au particle conjugates, lowering the solution pH modifies the DNA corona (Fig. 10) and induces self-assembly of the nanorod conjugates. In-situ LCEM with transient pH lowering revealed surprising differences in the interactions between the nanorod conjugates with different aspect ratio. Their assemblies are very different already at the dimer-stage. Short rods attach preferentially end-to-side (at angles close to 90°, Fig. 11). Long rods prefer side-to-side parallel dimers (Fig. 12). This difference in the dimer configuration is confirmed by a statistical analysis of in-situ LCEM movies (Fig. 13) and the aspect ratio dependent assembly motifs are consistent with calculated equilibrium structures for nanorods with pure van der Waals interaction (Fig. 13), confirming a pH-dependent interparticle potential that is tunable from repulsive (pH 7) to attractive (pH ≤5).
Overall our results showed that ssDNA-Au colloids represent a unique hybrid system for which self-assembly can be studied across a continuum of interactions via in-situ microscopy in solutions where the electron-induced acid spike leads to intermediate pH and hence an adjustable interparticle potential.
4. Novel experimental tools for in-situ microscopy of self-assembly and reconfiguration (Goals A.ii, B.i, B.ii) [Refs. 3, 5 & 8]: Combining programmable self-assembly, plasmonic light harvesting, and plasmon-mediated chemistry carries great potential for novel functional meso-architectures. LCEM can provide unprecedented insight into plasmon-mediated synthesis and self-organization. The focused electron beam represents a localized, evanescent source of white light that can excite LSPRs while also enabling real-time imaging in solution. Using such simultaneous excitation and imaging we identified a novel pathway toward plasmonic Ag nanoprisms (Fig. 14). Aggregation of Ag particles leads to small clusters (dimers, trimers). Due to intense near fields in nanogaps, preferential plasmonic reduction ($Ag^+ \rightarrow Ag^0$) between the particles causes the filling-in of the gaps to form Ag prisms. More broadly, plasmon-mediated metal deposition due to local field enhancements promises to become a powerful tool for the light-driven self-organization of plasmonic meso-architectures in solution.
5. Solvent driven reconfiguration of ordered DNA-nanoparticle superlattices (Goal B.iii) [Ref. (3)]: Substrate-supported assemblies of DNA-linked particles carry promise for future defense applications of nanocrystal-based smart materials. To address this emerging field, we extended our in-situ imaging capabilities to force microscopy (AFM). Using our standard oligos, we developed protocols to (i) functionalize particles and substrates with DNA, attach linkers, and assemble ordered 2D lattices (Fig. 15); and (ii) we identified minimally invasive imaging modes to enable damage-free imaging of 2D DNA-nanoparticle assemblies in solution.
We used in-situ AFM to observe superlattice reconfiguration induced via co-solvents (alcohols) (Figure 16),

previously investigated for suspended 3D supracrystals that showed reversible behavior. Probing EtOH-induced reconfiguration for supported superlattices linked to Au substrates via DNA ‘bonds’ we found incomplete reversibility manifesting itself in a striking hysteresis upon cycling. The lattice constant at 50% EtOH, for instance, depends on the pathway in which this state is reached (Figure 17). The hysteresis can be understood considering bonding to the support. Compliant ‘DNA bonds’ between the 2D assembly and the support counteract lattice parameter changes induced by changes in water:ethanol ratio, causing hysteresis in the reconfiguration of the superlattices. Our results are first in identifying key boundary conditions that affect the dynamic reconfiguration of supported DNA-linked 2D superlattices.

Training Opportunities: The project provided diverse opportunities for training and professional development to the participants, including personnel directly supported by this program (1 postdoc, 1 graduate student) and one postdoc who participated with other (UNL-internal) sources of support. The participating graduate student, Mr. Bo Zhang received training from the PIs as well as extensive hands-on experience in nanoparticle synthesis, the chemistry of DNA-nanoparticle conjugation, as well as characterization approaches for DNA-nanoparticle conjugates and superlattices. He was trained in transmission electron microscopy and atomic force microscopy, and the planning and realization of complex in-situ experiments. A postdoctoral fellow, Dr. Muhua Sun, worked for 8 months on the project during which time she received training from the PIs in advanced liquid cell electron microscopy techniques. A second postdoc (supported by UNL funds), Dr. Mikhail Shekhirev, received training in DNA-nanoparticle conjugation and in advanced atomic force microscopy approaches for in-situ surface microscopy. All participants were mentored by the PIs in the complex protocols required to analyze real-time and in-situ data streams, as well as the dissemination of results via posters, oral technical presentations, and manuscripts for publication.

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Results Dissemination: A. Publications:
Reference (1): P. Sutter, B. Zhang, and E. Sutter, “Radiation damage during in situ electron microscopy of DNA-mediated nanoparticle assemblies in solution”, Nanoscale 10, 12674 (2018) [DOI: 10.1039/C8NR04087G]
Reference (2): E. Sutter, B. Zhang, and P. Sutter, “In-Situ Electron Microscopy of the Self-Assembly of Single-Stranded DNA-Functionalized Au Nanoparticles in Aqueous Solution”, Nanoscale 11, 34 (2019). [DOI: 10.1039 /C8NR08421A]
Reference (3): M. Shekirev, E. Sutter and P. Sutter, “In-Situ Atomic Force Microscopy of the Reconfiguration of On-Surface Self-Assembled DNA-Nanoparticle Superlattices”, Advanced Functional Materials 29, 1806924 (2019). [DOI: 10.1002/adfm.201806924]
Reference (4): E. Sutter, “Colloidal Nanostructures: In-Situ Electron Microscopy of Plasmon-Mediated Synthesis, Chemistry and Self-Assembly”, Microscopy and Microanalysis 25, Suppl. S2, 1404 (2019). [DOI: 10.1017 /S143192761900775X]
Reference (5): M. Sun, Y. Li, B. Zhang, C. Argyropoulos, P. Sutter, and E. Sutter, “Plasmonic Effects on the Growth of Ag Nanocrystals in Solutions”, Langmuir 36, 2044 (2020). [DOI: 10. 1021/acs.langmuir.9b03765]
Reference (6): E. Sutter, B. Zhang and P. Sutter, “DNA-Mediated Three-Dimensional Assembly of Hollow Au-Ag Alloy Nanocages as Plasmonic Crystals”, ACS Appl. Nano Mater. 3, 8068 (2020). [DOI: 10.1021/acsanm.0c01528] ACS Appl. Nano Mater. Cover [https://pubs.acs.org/toc/aanmf6/3/8]
Reference (7): E. Sutter, B. Zhang, and P. Sutter, “Single-Strand DNA-Nanorod Conjugates - Tunable Anisotropic Colloids for On-Demand Self-Assembly”, J. of Colloid and Interface Science 586, 847 (2021).[DOI:10.1016/j.jcis. 2020.11.009]
Reference (8): (Invited Article) P. Sutter, and E. Sutter, “Real-Time Electron Microscopy of Nanocrystal Synthesis, Transformations, and Self-Assembly in Solution”, Accounts of Chem. Research 54, 11 (2021), Special issue on “Transformative Inorganic Nanocrystals”. [DOI: 10.1021/acs.accounts.0c00678]

B. Invited Talks
(1) Eli Sutter, “Colloidal Nano-structures: In-Situ Electron Microscopy of Plasmon-Mediated Synthesis, Chemistry and Self-Assembly”, 21st American Conference on Crystal Growth and Epitaxy (ACCGE-21), Santa Fe (NM), August 1, 2017 (Invited).
(2) Eli Sutter, “In-situ Electron Microscopy of Synthesis, Chemistry and Self-Assembly of Colloidal Nanostructures”, International Workshop on Liquid Phase Electron Microscopy, Eindhoven (Netherlands), September 19, 2017 (Invited).
(3) Eli Sutter, “In-situ Electron Microscopy of Plasmon Mediated Synthesis, Chemistry and Self-Assembly of Colloidal Nanostructures”, Pharmaceutical Sciences, University of Nebraska Medical Center, January 19, 2018 (Invited).
(4) Eli Sutter, “Design of New Materials in Solution: Insights from In-Situ Electron Microscopy”, International Conference on Low Dimensional Quantum Materials, Snowbird (UT), March 13, 2018 (Invited).
(5) Peter Sutter, “Real-Time Microscopy of Electron-Beam Induced Transformations: From 2D Materials to Anisotropic Nano-Colloids”, 2017 MRS Spring Meeting, Symposium MA03—Directed Matter—Atom-by-Atom Assembly with Electron Beams and Scanning Probes, Phoenix (AZ), April 3, 2018 (Invited).
(6) Eli Sutter, “Design of New Materials in Solution: Insights from In-Situ Electron Microscopy”, International Materials Research Congress, Cancun (Mexico), August 20, 2018 (Invited).
(7) E. Sutter, “Real-Time Microscopy of Electron-Beam Induced Transformations: From 2D Materials to Anisotropic Nano-Colloids”, International Nanotechnology Conference, Vienna, Austria, September 3-5, 2018 (Invited).
(8) E. Sutter, “Design of New Materials: Insights from In-Situ Electron Microscopy”, University of Nebraska-Lincoln Conference for Undergraduate Women in Physical Sciences (WoPhyS '18), October 12, 2018 (Keynote).
(9) E. Sutter, “Self-Assembly of Nanocrystals in Solution: Insights from In-Situ Electron Microscopy”, MRS Spring Meeting, Phoenix (AZ), April 24, 2019 (Invited).
(10) E. Sutter, “Colloidal Nanostructures: In-Situ Electron Microscopy of Plasmon-Mediated Synthesis, Chemistry and Self-Assembly”, Microscopy and Microanalysis Meeting, Portland OR, August 6, 2019 (Invited).
(11) M. Shekirev, E. Sutter, P. Sutter, “In-Situ Atomic Force Microscopy of DNA-Mediated Nanoparticle Assemblies in Solution”, MRS Fall Meeting, Boston (MA), November 26, 2018.
(12) E. Sutter, “Designing New Materials: In-Situ Imaging, Measurements, and Manipulation in the Electron Microscope”, University of Notre Dame, South Bend (IN), November 5, 2019. (Invited)

Honors and Awards: Nothing to Report

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PARTICIPANTS:

Participant Type: PD/PI
Participant: Eli Sutter
Person Months Worked: 9.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Co PD/PI
Participant: Peter Sutter
Person Months Worked: 9.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: Muhua Sun
Person Months Worked: 8.00 **Funding Support:**
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Participant Type: Graduate Student (research assistant)
Participant: Bo Zhang
Person Months Worked: 15.00 **Funding Support:**
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Article Title: Radiation damage during in situ electron microscopy of DNA-mediated nanoparticle assemblies in solution
Authors: Peter Sutter, Bo Zhang, Eli Sutter
Keywords: DNA, Self-Assembly, Radiation Damage, In-Situ Microscopy
Abstract: Oligonucleotide–nanoparticle conjugates, also called programmable atom equivalents, carry promise as building blocks for self-assembled colloidal crystals, reconfigurable or stimuli responsive functional materials, as well as bio-inspired hierarchical architectures in wet environments. In situ studies of the DNA-mediated self-assembly of nanoparticles have so far been limited to reciprocal space techniques. Liquid-cell electron microscopy could enable imaging such systems with high resolution in their native environment but to realize this potential, radiation damage to the oligonucleotide linkages needs to be understood and conditions for damage-free electron microscopy identified. Here, we analyze in situ observations of DNA-linked two-dimensional nanoparticle arrays, along with control experiments for different oligonucleotide configurations, to identify the mechanisms of radiation damage for ordered superlattices of DNA–nanoparticle conjugates. In a biological context, the results

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Article Title: electron microscopy of the self-assembly of single-stranded DNA-functionalized Au nanoparticles in aqueous solution
Authors: Eli Sutter, Bo Zhang, Stephan Sutter, Peter Sutter
Keywords: single-stranded DNA, nanoparticles, self-assembly, in-situ microscopy
Abstract: Solution-phase self-assembly of DNA-functionalized nanoparticles into mesoscale structures is a promising strategy for creating functional materials from nanocrystal building blocks. The predominant approach has been the use of Watson–Crick base pairing between complementary bases in designated ‘sticky ends’ to trigger programmable self-assembly into ordered superlattices. Here we demonstrate the ordered self-assembly of Au nanoparticles conjugated with single-stranded (ss) DNA in acidic solutions. Au nanoparticles functionalized with thiolated ssDNA are protected against coalescence and the DNA conformation undergoes significant modifications at low pH, which can be associated with the protonation of adenine bases and the formation of a parallel poly-adenine duplex, which govern the interaction between ssDNA–Au nanoparticle conjugates. In situ liquid cell electron microscopy enables real-time imaging of the self-assembly process and the identification of key characteristics, such as t

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Article Title: In Situ Atomic Force Microscopy of the Reconfiguration of On-Surface Self-Assembled DNA-Nanoparticle Superlattices
Authors: Mikhail Shekhirev, Eli Sutter, Peter Sutter
Keywords: DNA, in-situ microscopy, nanoparticles, self-assembly, smart materials
Abstract: The ability to dynamically reconfigure superlattices in response to external stimuli is an intriguing prospect for programmable DNA?guided nanoparticle (NP) assemblies, which promises the realization of “smart” materials with dynamically adjustable interparticle spacing and real?time tunable properties. Existing in situ probes of reconfiguration processes have been limited mostly to reciprocal space methods, which can follow larger ordered ensembles but do not provide access to real?space pathways and dynamics. Here, in situ atomic force microscopy is used to investigate DNA?linked NP assemblies and their response to external stimuli, specifically the contraction and expansion of on?surface self?assembled monolayer superlattices upon reversible DNA condensation induced by ethanol. In situ microscopy allows observation and quantification of key processes in solution, e.g., lattice parameter changes, defects, and monomer displacements in small groups of NPs. The analysis of imaging data
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Authors: E. Sutter
Keywords: Self-Assembly, Nanoparticles, In-situ Microscopy, Surface Plasmon Resonance, Synthesis
Abstract: Real-time transmission electron microscopy can be used to follow the behavior and measure the properties of nanostructures over a wide range of environmental conditions with resolution down to the atomic scale. Liquid-cell electron microscopy (LCEM), for instance, is the only technique that allows direct imaging of nanometer-scale processes in liquids. It has been successfully applied to imaging various processes in liquids, solutions, and colloidal suspensions that were typically investigated ex-situ on samples taken at different process stages, or in some cases in-situ using reciprocal space techniques. Here we will illustrate the power of in-situ liquid-cell electron microscopy imaging to probe complex solution-phase processes in real space. Examples include plasmon-mediated colloidal synthesis of anisotropic nanostructures and the self-assembly of nanocrystal superstructures in solution.
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Authors: Eli Sutter, Bo Zhang, Peter Sutter
Keywords: Hollow noble metal nanostructures, Au-Ag nanocages, DNA-guided self-assembly, cathodoluminescence, plasmonics
Abstract: Oligonucleotide-nanoparticle conjugates carry promise as building blocks for stimuli responsive, reconfigurable functional materials in wet environments. Here, we demonstrate the self-assembly of DNA-linked hollow Au-Ag alloy nanocages into supracrystals in aqueous solution. Initially, the surface of Ag nanoparticles is modified by depositing porous layers of gold, which allows functionalization of the particles with thiolated DNA following established approaches for functionalizing Au nanoparticles. Galvanic replacement in aqueous Au-ion solution transforms the solid DNA conjugates into hollow, DNA terminated Au-Ag alloy nanocages that can be linked into three-dimensional, long-term stable nanocage arrays by self-assembly during slow cooling. Cathodoluminescence of individual hollow nanostructure assemblies demonstrates collective plasmonic behavior with strong localized surface plasmon resonance enhancement at the surface of the supracrystals. Our results identify an approach toward
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Authors: Muhua Sun, Ying Li, Bo Zhang, Christos Argyropoulos, Peter Sutter, Eli Sutter
Keywords: liquid cell electron microscopy, anisotropic nanostructures, plasmonics, cathodoluminescence
Abstract: The light-stimulated transformation of ensembles of spherical nanoparticles into anisotropic metal nanostructures mediated by localized surface plasmon resonance (LSPR) excitation is an elegant way of synthesizing triangular silver nanoprisms with extraordinary control over size and shape. Usually the transformation occurs in oxidizing environments along a pathway that involves the oxidative etching of small pre-existing Ag seeds, followed by plasmon-mediated reduction of the resulting Ag ions and Ag0 incorporation into the anisotropic nanocrystals. Here, we investigate pathways toward Ag nanoprisms from initially homogeneous AgNO3 solutions held at reducing conditions. Observations by in-situ electron microscopy show that reducing environments and high Ag precursor concentrations in the presence of sodium citrate favor two alternative transformation routes of initial spherical nuclei into anisotropic nanoprisms: (i) the aggregation of spherical nanoparticles and plasmon-mediated conve
Distribution Statement: 2-Distribution Limited to U.S. Government agencies only; report contains proprietary info
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RPPR Final Report
as of 16-Sep-2021

URL: https://unlcms.unl.edu/engineering/mme/eli-sutter/welcome
Date Received: 30-Aug-2018
Title: Sutter Group Research Web Site
Description: Web Site including information on research supported by this project, dissemination of results, acknowledgement of ARO funding.

Partners

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I certify that the information in the report is complete and accurate:
Signature: Eli Sutter
Signature Date: 6/7/21 2:34PM

Final Report – June 2021

In-Situ Electron Microscopy of DNA-Guided Self-Assembly and Reconfiguration of 3D Nanocrystal Superlattices

Principal Investigators:
Prof. Eli Sutter, Mechanical & Materials Engineering, University of Nebraska-Lincoln
Prof. Peter Sutter, Electrical & Computer Engineering, University of Nebraska-Lincoln

Army Research Office Program: Engineering Sciences Directorate, Materials Science Division, Materials Design Program

Technical Point of Contact: Dr. Evan Runnerstrom; e-mail: evan.l.runnerstrom.civ@mail.mil, (919) 549-4259

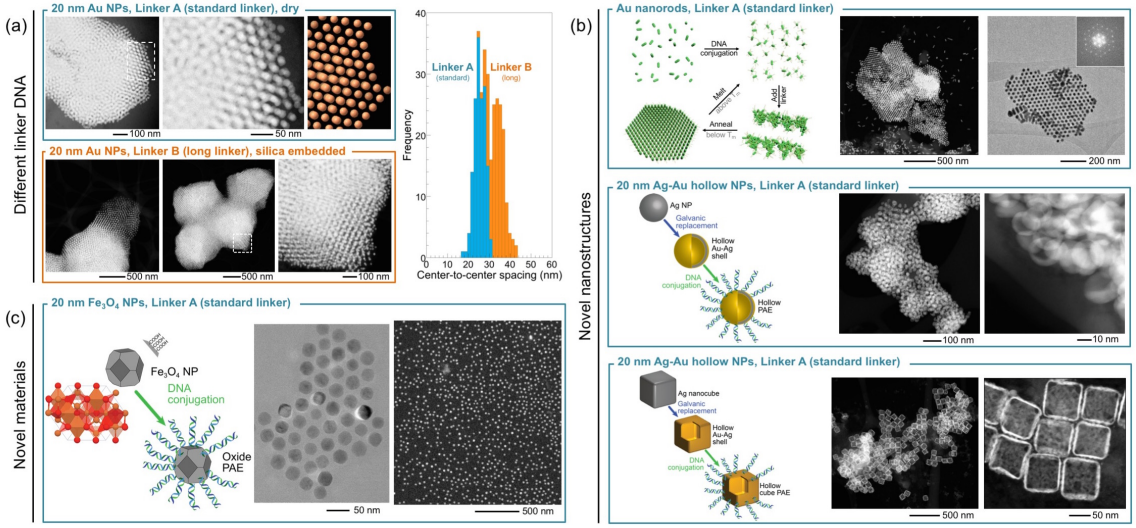

Figure 1. Examples of the library of DNA-nanostructure conjugates developed by this program. (a) 20 nm Au nanoparticles (NPs) conjugated with our standard particle-bound DNA (see Figure 2 below) and linked with different linker DNA: Short Linker (A), Long Linker (B). In both cases, use of linkers with self-complementary recognition base sequence leads to crystallization in face-centered cubic (fcc) structure. **(b)** Conjugation and self-assembly of different nanostructures into ordered superlattices: Au nanorods, 20 nm hollow Ag-Au nanocages and Ag-Au nanocubes. **(c)** Non-noble metal programmable atom equivalents, which were pursued to reduce radiation damage to oligonucleotides during *in-situ* liquid cell (S)TEM.

Oligos	Base sequences
Particle-bound DNA	5'-HS-A_{10}-AAG ACG AAT ATT TAA CAA-3'
Linker A (standard linker, 4-base recognition region)	3'-TTC TGC TTA TAA ATT GTT-A-GCGC-5'
Dummy Linker A (no recognition region)	3'-TTC TGC TTA TAA ATT GTT-5'
Linker B (long linker)	3'-TCT GCT TAT AAA TTG TTA TTT TTT TTT TTT ACT GAG CAG CAC TGA TTT TTT TTT TTT T-A-GCGC-5'
Linker B duplexer strand	5'-AAA AAA AAA AAA TGA CTC GTC GTG ACT AAA AAA AAA AAA A-3'
Linker C (standard linker, 6-base recognition region)	3'-TTC TGC TTA TAA ATT GTT-A-GCGCGC-5'

Figure 2. Summary of particle-bound and linker DNA base sequences. Nanoparticles and other nanostructures were typically conjugated with a standard **Particle-bound DNA**, a thiolated oligonucleotide to which different linker DNA strands could be hybridized: **Linker A:** Standard/short linker with 4-base 'sticky end' recognition sequence; **Dummy Linker A:** Standard/short linker without a recognition sequence. **Linker B:** Long linker, with optional **duplexer strand**; **Linker C:** Standard/short linker with extended 6-base recognition sequence.

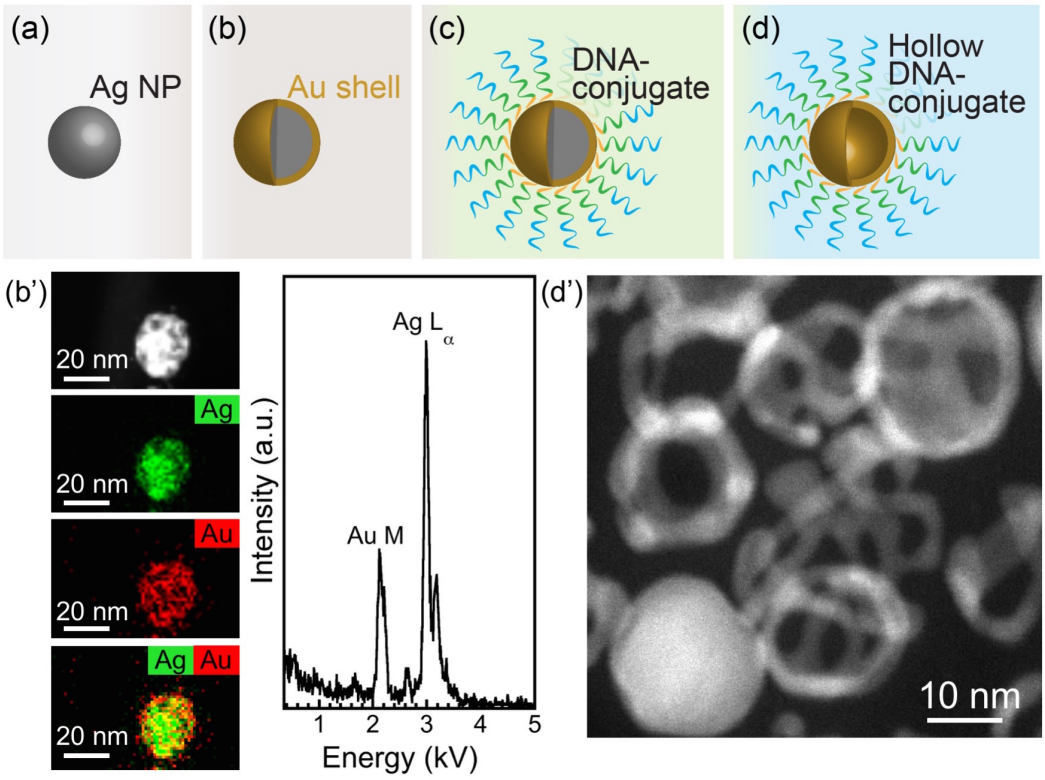

Figure 3. Schematic summary of the process developed to produce hollow DNA-nanocage conjugates. (a) Starting Ag nanoparticles (NPs). **(b)** Deposition of a gold shell on the surface of the Ag nanoparticles. **(c)** Formation of DNA-Ag-Au core-shell nanoparticles conjugates. **(d)** Hollowing out of the conjugates using a galvanic replacement reaction. **(b')** HAADF-STEM and EDS chemical maps of a nanoparticle showing the distribution of Ag and Au immediately after immersion of the Ag nanoparticles in 100 μM $HAuCl_4$ aqueous solution. The EDS spectrum of the nanoparticle shows the presence of ~16 at.% gold. **(d')** HAADF-STEM images of Au-Ag nanocages following the conjugation of the initial Ag-Au core-shell structures with ssDNA (5'-HS-A_{10}-AAG ACG AAT ATT TAA CAA-3') and their complete hollowing by galvanic replacement.

Reference: E. Sutter, B. Zhang, and P. Sutter, "*DNA-Mediated Three-Dimensional Assembly of Hollow Au-Ag Alloy Nanocages as Plasmonic Crystals*", ACS Appl. Nano Mater. **3**s 8068 (2020).

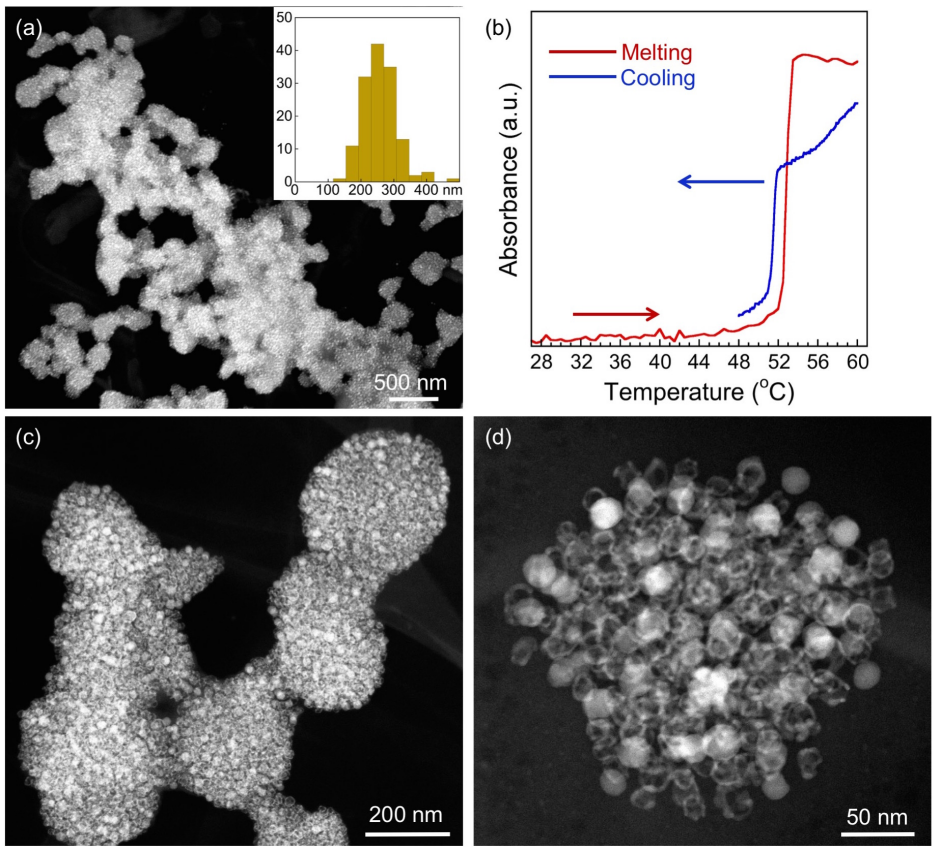

Figure 4. Melting/crystallization and morphology of DNA-linked nanocage assemblies. (a) Low-magnification HAADF STEM image of 3D DNA-Au-Ag nanocage assemblies. Inset: Size distribution of the supracrystals formed by heating and slow cooling below the melting temperature. **(b)** UV-VIS spectroscopy of the absorbance at a fixed wavelength (410 nm) during slow heating (red) and cooling (blue) of suspensions of DNA-(Au-Ag) nanocage conjugates with added functional linker DNA in aqueous solutions of 10 mM phosphate buffer and 0.5 M NaCl. **(c)** Close-up view showing the 3D assemblies of hollow cages. **(d)** Small close-packed 3D assembly showing the morphology and arrangement of the nanocages.

Reference: E. Sutter, B. Zhang, and P. Sutter, "*DNA-Mediated Three-Dimensional Assembly of Hollow Au-Ag Alloy Nanocages as Plasmonic Crystals*", ACS Appl. Nano Mater. **3**, 8068 (2020).

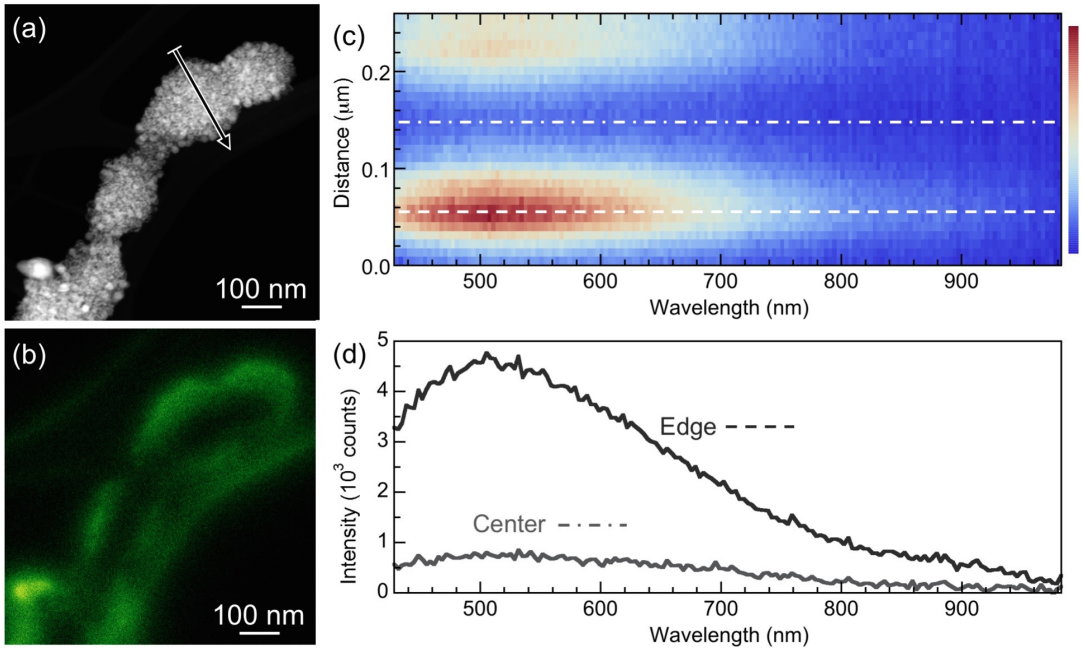

Figure 5. Cathodoluminescence spectroscopy of the surface plasmon resonance of DNA-linked Au-Ag nanocage assemblies. (a) STEM image and **(b)** panchromatic STEM-cathodoluminescence (CL) map of a group of four assemblies of DNA-Au-Ag nanocage conjugates. The coupling of the electron beam to the LSPR is strongest near the periphery of the assemblies. **(c)** Hyperspectral linescan comprising full luminescence spectra across the assembly along the line marked in (a). **(d)** Individual local luminescence spectra obtained with the exciting electron beam placed at the edge and near the center of the assembly (at positions indicated in (c)).

Reference: E. Sutter, B. Zhang, and P. Sutter, "*DNA-Mediated Three-Dimensional Assembly of Hollow Au-Ag Alloy Nanocages as Plasmonic Crystals*", ACS Appl. Nano Mater. **3**, 8068 (2020).

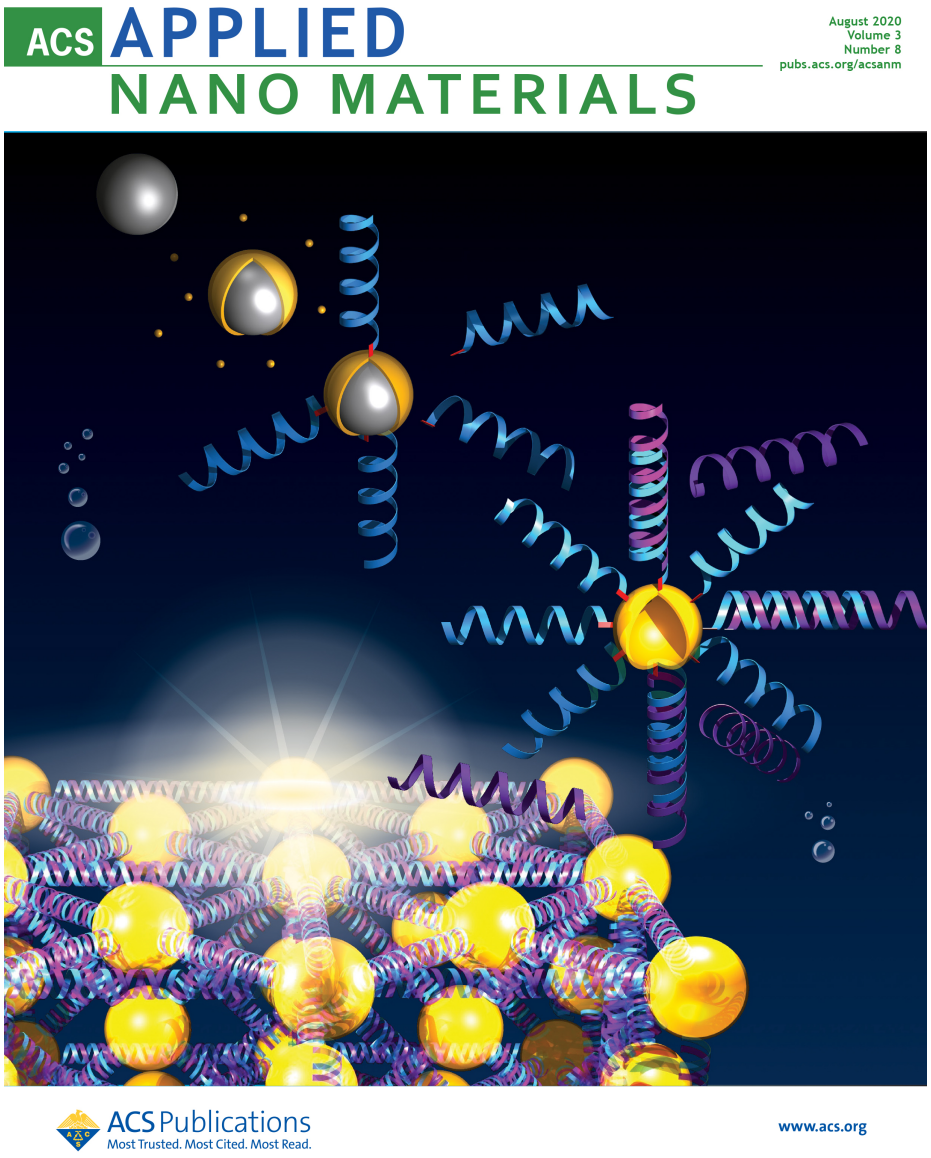

Figure 6. ACS Applied Nano Materials journal cover
[https://pubs.acs.org/toc/aanmf6/3/8].

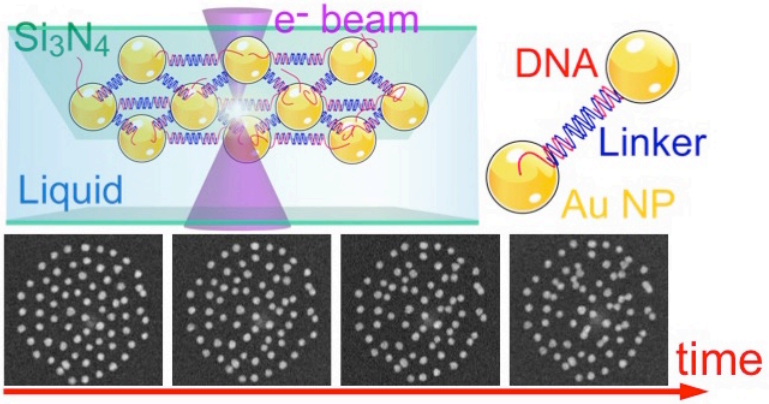

Figure 7. *In-situ* microscopy of radiation damage to DNA in solution, and identification of low-dose imaging conditions for damage-free *in-situ* imaging of DNA-linked nanoparticle assemblies. Schematic of the liquid cell electron microscopy of specifically developed 2D superlattices of DNA-Au nanoparticle conjugates, and time-lapse STEM image series illustrating the early stages of radiation damage to DNA.

Reference: P. Sutter, B. Zhang, and E. Sutter, "*Radiation damage during in situ electron microscopy of DNA-mediated nanoparticle assemblies in solution"*, Nanoscale **10**, 12674 (2018).

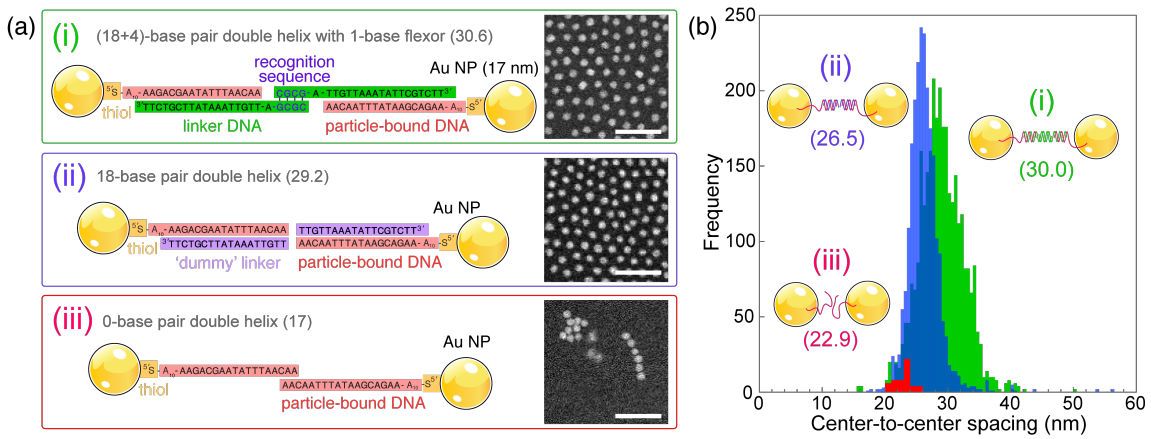

Figure 8. Identification of DNA radiation damage pathways using *in-situ* liquid cell electron microscopy on assemblies of 17 nm Au particles with different DNA functionalization. (a) Schematic representation of three different functionalizations: **(i)** Particle-bound DNA and functional Linker A with 4-base recognition sequence; **(ii)** Particle-bound DNA transformed into a double helix by hybridization with Dummy linker A; and **(iii)** particle-bound ssDNA only (no linker). Numbers in brackets are the predicted center-to-center separations (in nm) for 17 nm particles spaced by the cumulative length of double helical segments (assuming a length of 0.34 nm per base pair). Images show particle configurations observed by *in-situ* electron microscopy in solution for each case. Scale bars: 100 nm. **(b)** Statistical analysis of center-to-center particle spacing from measurements by *in-situ* electron microscopy in solution. Case (iii) is based on observations of small clusters (as shown in (a)), whereas (i) and (ii) are derived from larger 2D assemblies. Numbers in brackets are measured mean center-to-center separations (in nm).

Reference: P. Sutter, B. Zhang, and E. Sutter, "*Radiation damage during in situ electron microscopy of DNA-mediated nanoparticle assemblies in solution*", Nanoscale **10**, 12674 (2018).

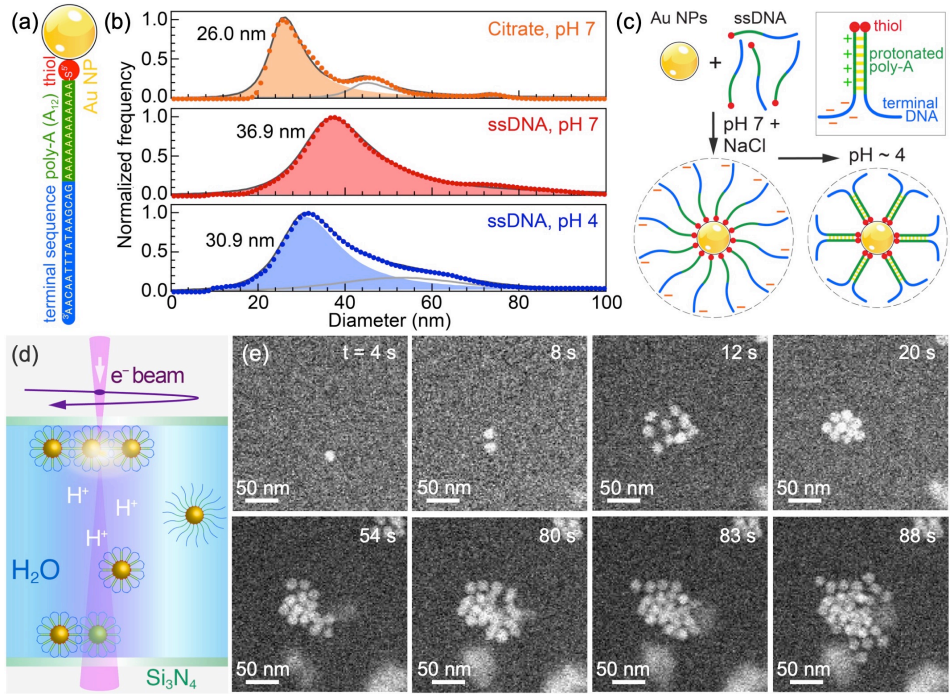

Figure 9. Directed self-assembly at low pH of single-strand DNA (ssDNA) conjugated nanocrystals. (a) Schematic of the ssDNA nanoparticle conjugates and oligonucleotide base sequence. **(b)** Hydrodynamic diameter of Au nanoparticles with different surface terminations at pH 7 and 4. **(c)** Tunable morphology and interaction of ssDNA-Au nanoparticle conjugates. Neutral pH: Extended DNA strands, large hydrodynamic diameter, electrostatic repulsion between charged monomers. Acidic pH: Parallel duplex of protonated adenine bases and backfolded terminal sequence, reduced diameter, self-assembly of charge-neutral nanocrystals. **(d)** Schematic of in-situ HAADF-STEM in a liquid cell enclosing bulk aqueous solution between ultrathin silicon nitride membranes. Triggered by the electron beam-induced acid-spike, the nanocrystals tend to assemble near both the top and bottom membranes of the cell. **(e)** Real-time STEM imaging of the self-assembly of ssDNA-conjugated Au nanocrystals in solution.

Reference: E. Sutter, B. Zhang, and P. Sutter, "In-Situ Electron Microscopy of the Self-Assembly of Single-Stranded DNA-Functionalized Au Nanoparticles in Aqueous Solution", Nanoscale **11**, 34 (2019).

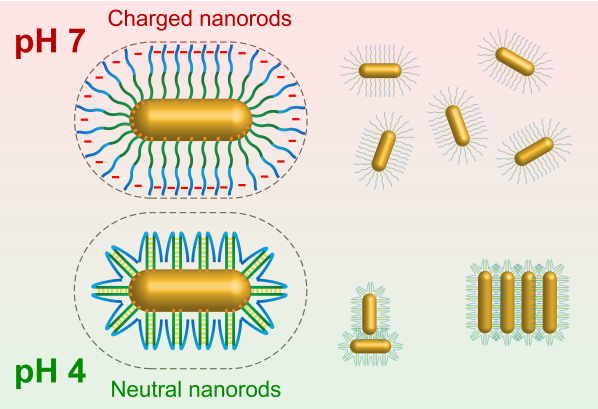

Figure 10. Tunable interaction of single-strand DNA (ssDNA) Au nanorod conjugates. Neutral pH: Extended DNA strands, large hydrodynamic diameter, electrostatic repulsion between charged monomers. **Acidic pH:** Poly-A parallel duplex of protonated adenine bases and backfolded terminal sequence, reduced hydrodynamic diameter, van der Waals attraction and self-assembly of charge-neutral nanorods.

Reference: E. Sutter, B. Zhang, and P. Sutter, "Single-Strand DNA-Nanorod Conjugates - Tunable Anisotropic Colloids for On-Demand Self-Assembly", J. of Colloid and Interface Science **586**, 847 (2021).

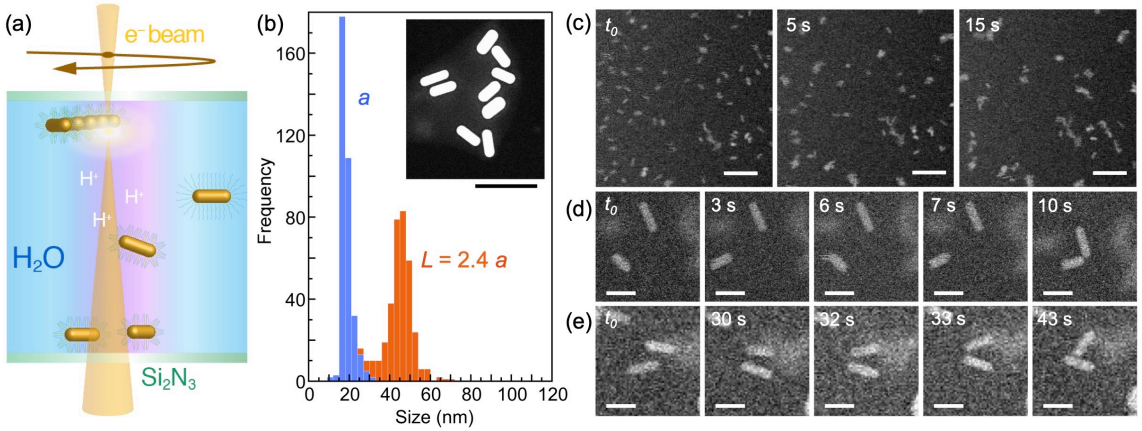

Figure 11. ***In-situ*** **imaging of the self-assembly of ssDNA-short Au nanorod conjugates in solution. (a)** Schematic of *in-situ* HAADF-STEM using a microfabricated liquid cell with bulk aqueous solution enclosed between ultrathin (electron transparent) silicon nitride membranes. **(b)** Size distribution (diameter *a*, length *L*) of short Au nanorods (aspect ratio *L*/a = 2.4) conjugated with ssDNA and self-assembled at low pH. Inset: STEM image, scale bar: 100 nm. **(c)** Real-time *in-situ* HAADF-STEM of ssDNA-conjugated short Au nanorods in solution. Elapsed times are shown relative to the initial frame (time t_0). Scale bars: 200 nm. **(d), (e)** Time-lapse STEM image series showing the formation of characteristic short nanorod dimer configurations. Scale bars: 50 nm.

Reference: E. Sutter, B. Zhang, and P. Sutter, "Single-Strand DNA-Nanorod Conjugates - Tunable Anisotropic Colloids for On-Demand Self-Assembly", J. of Colloid and Interface Science **586**, 847 (2021).

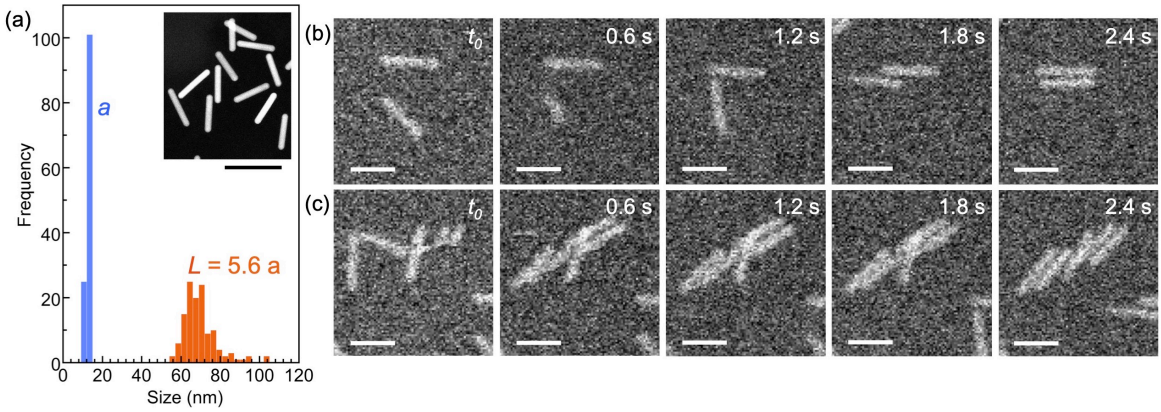

Figure 12. *In-situ* imaging of the self-assembly of ssDNA-long Au nanorod conjugates in solution. (a) Size distribution of long Au nanorods (aspect ratio L/a = 5.6). Inset: STEM image, scale bar: 100 nm. **(b), (c)** Time-lapse STEM image series showing the formation of characteristic long nanorod dimers and larger oligomers. Scale bars: 50 nm.

Reference: E. Sutter, B. Zhang, and P. Sutter, "Single-Strand DNA-Nanorod Conjugates - Tunable Anisotropic Colloids for On-Demand Self-Assembly", J. of Colloid and Interface Science **586**, 847 (2021).

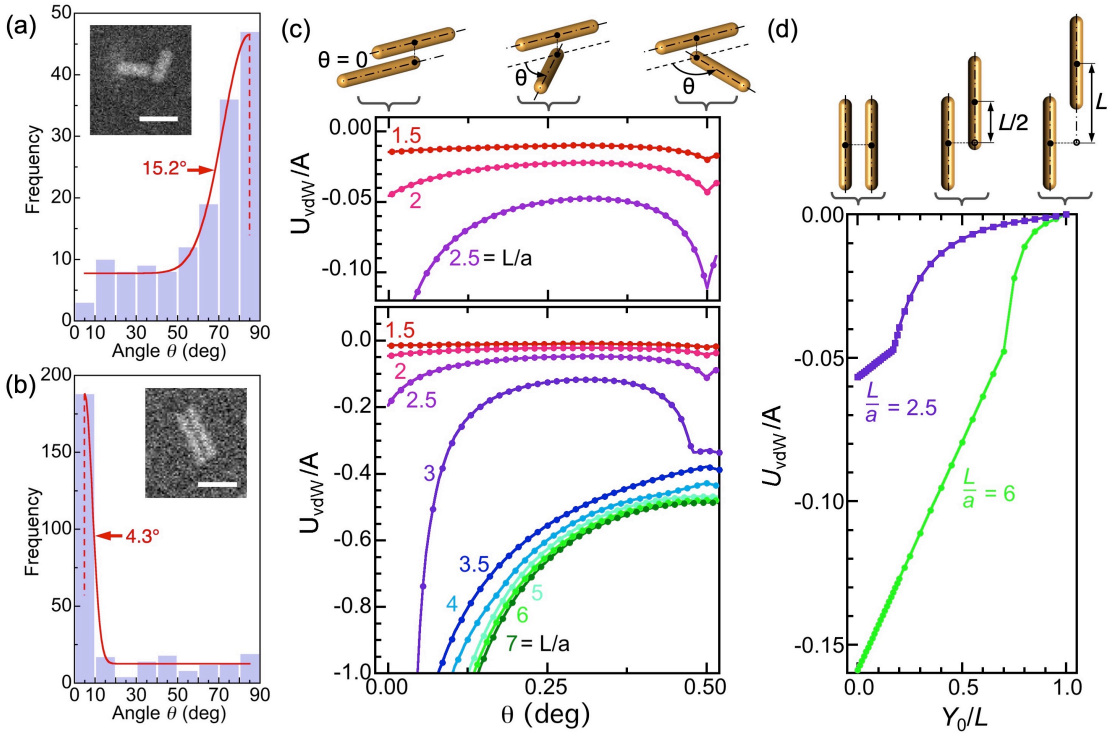

Figure 13. Aspect-ratio dependent preferred motifs of van der Waals nanorod dimers. (a) Histogram of dimer orientations of short ssDNA-Au nanorod conjugates (L/a = 2.4) observed by *in-situ* microscopy in solution. Inset: Characteristic example of a dimer (scale bar: 50 nm). **(b)** Histogram of dimer orientations of long ssDNA-Au nanorod conjugates (L/a = 5.6) observed by *in-situ* microscopy in solution. Inset: Characteristic example of a dimer (scale bar: 50 nm). Solid lines in (a) and (b) are Gaussian fits to the peaks in the distributions, labeled with their respective half-width at half maximum. **(c)** Calculated energy U_{vdW} (relative to the material-dependent Hamaker constant, A) of nanorod dimers as a function of the relative orientation angle θ for different rod aspect ratios L/a ranging from 1.5 to 7. **(d)** Computed energy landscape for aligned but axially shifted nanorods (with relative shift Y_0) for two different aspect ratios L/a, close to those in our experiments.

Reference: E. Sutter, B. Zhang, and P. Sutter, "Single-Strand DNA-Nanorod Conjugates - Tunable Anisotropic Colloids for On-Demand Self-Assembly", J. of Colloid and Interface Science **586**, 847 (2021).

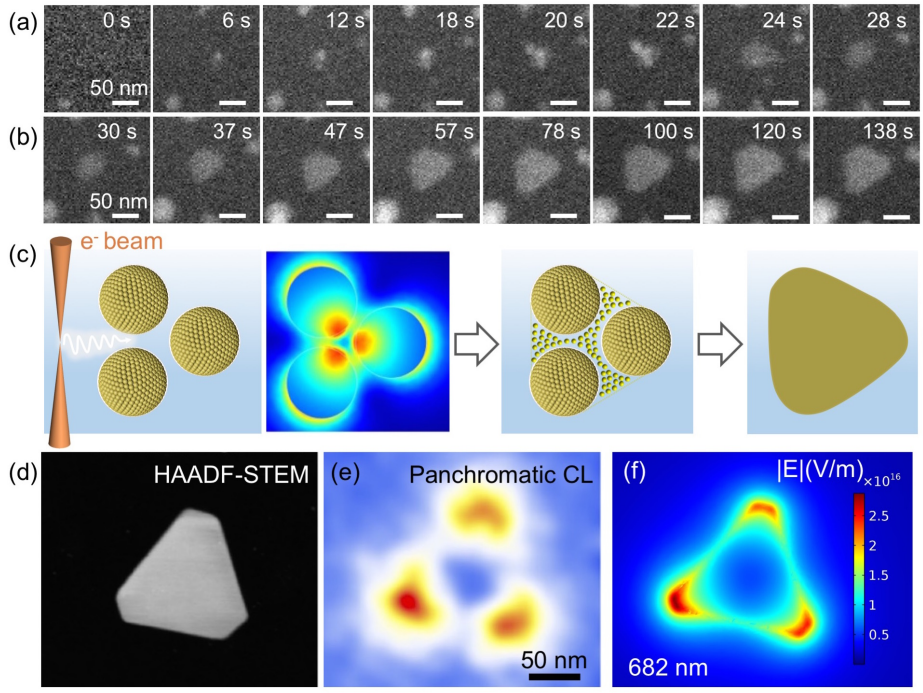

Figure 14. *In-situ* liquid cell electron microscopy of plasmon-mediated nanoprism growth in aqueous $AgNO_3$/sodium citrate solutions. (a) Time-lapse sequence of *in-situ* HAADF-STEM images showing the formation of a small plate-like Ag nanocrystal through interaction of small Ag nanoparticles during the initial growth stage (t = 0 s to 28 s) in aqueous solution containing $AgNO_3$ and sodium citrate. **(b)** *In-situ* microscopy of the subsequent evolution: Following the formation of an anisotropic nucleus from closely spaced spherical particles, the Ag plate grows shape-invariant as a truncated triangular nanoprism. **(c)** Schematic illustration of the newly established mechanism of plasmon-mediated growth of Ag nanostructures. The electric field of the electron probe excites the localized surface plasmon resonance (LSPR) of Ag nanoparticles and causes locally enhanced near-fields that promote the preferential deposition of Ag in areas between closely spaced particles and transformation of small nanoparticle clusters into anisotropic truncated triangular Ag nanoprisms followed by shape-invariant growth. **(d)** HAADF-STEM image of a truncated Ag nanoprism. **(e)** Panchromatic STEM-CL map showing strongest coupling to the LSPR (i.e., highest luminescence intensity) when the electron beam is positioned near the corners of the Ag prism. **(f)** Simulated field distribution of the dipolar plasmon resonance mode for the Ag nanoprism in (d).

Reference: M. Sun, Y. Li, B. Zhang, C. Argyropoulos, P. Sutter, and E. Sutter, *"Plasmonic Effects on the Growth of Ag Nanocrystals in Solutions"*, Langmuir **36**, 2044 (2020).

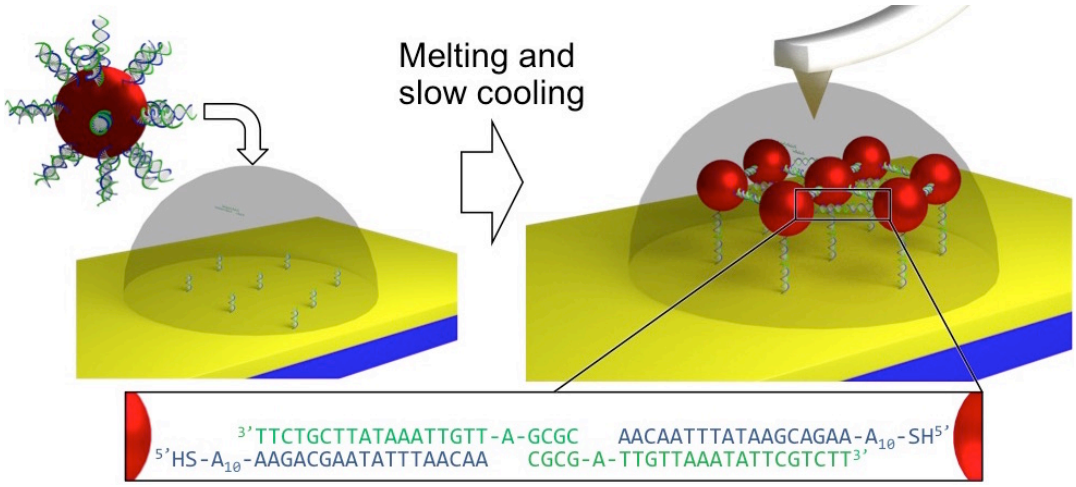

Figure 15. *In-situ* atomic force microscopy of self-assembly and reconfiguration of DNA-directed surface-supported nanoparticle superlattices in solution. Both Au thin film substrates and 20 nm Au nanoparticles were conjugated with particle-bound DNA, followed by hybridization with **Linker A** (see Figure 2). Annealing above the melting point of the 4-base 'sticky end' and slow cooling produced well-ordered (polycrystalline) 2D assemblies, which were imaged *in-situ* in the solution using minimally invasive (i.e., ultralow force) atomic force microscopy.

Reference: M. Shekhirev, E. Sutter, and P. Sutter, "*In Situ Atomic Force Microscopy of the Reconfiguration of On-Surface Self-Assembled DNA-Nanoparticle Superlattices*", Advanced Functional Materials **29**, 1806924 (2019).

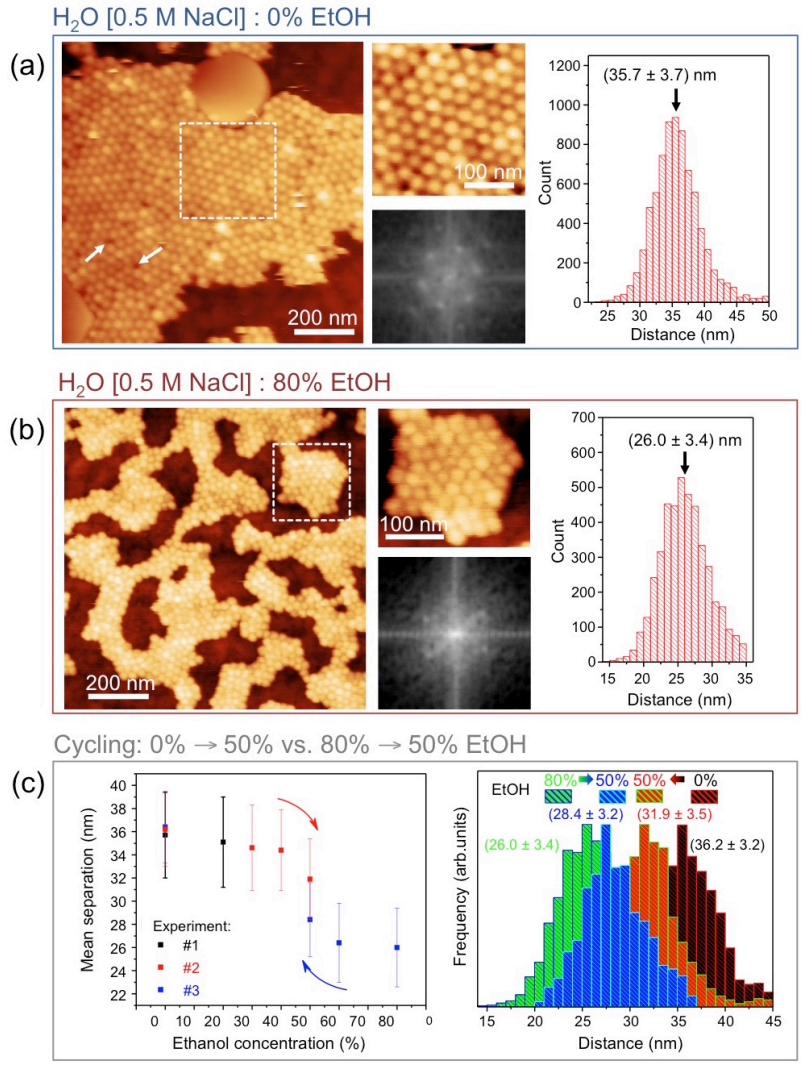

Figure 16. *In-situ* AFM imaging of the reconfiguration of DNA-linked 2D nanoparticle superlattices by adjusting the water:ethanol ratio. (a) Monomer-resolved *in-situ* force microscopy of a DNA-mediated monolayer (2D) superlattice of 20 nm Au particles in aqueous solution (0% ethanol). Mean center-to-center spacing: 35.7 nm; hexagonal lattice symmetry; standard particle-bound DNA; **Linker A** (short linker with 4-base recognition sequence, see Figure 2). **(b)** 2D DNA-Au nanoparticle superlattice following contraction initiated by adjusting the ethanol content to 80%. Previously continuous domains fracture into single-crystalline grains by bond breaking at low-coordination grain boundaries; pores open up due to the lattice contraction. Mean center-to-center spacing: 26.0 nm. **(c)** Hysteresis in the lattice expansion/contraction: the lattice constant at 50% ethanol depends on the pathway to that state (0% → 50%, or 0% → 80% → 50%).

Reference: M. Shekhirev, E. Sutter, and P. Sutter, "*In Situ Atomic Force Microscopy of the Reconfiguration of On-Surface Self-Assembled DNA-Nanoparticle Superlattices*", Advanced Functional Materials **29**, 1806924 (2019).

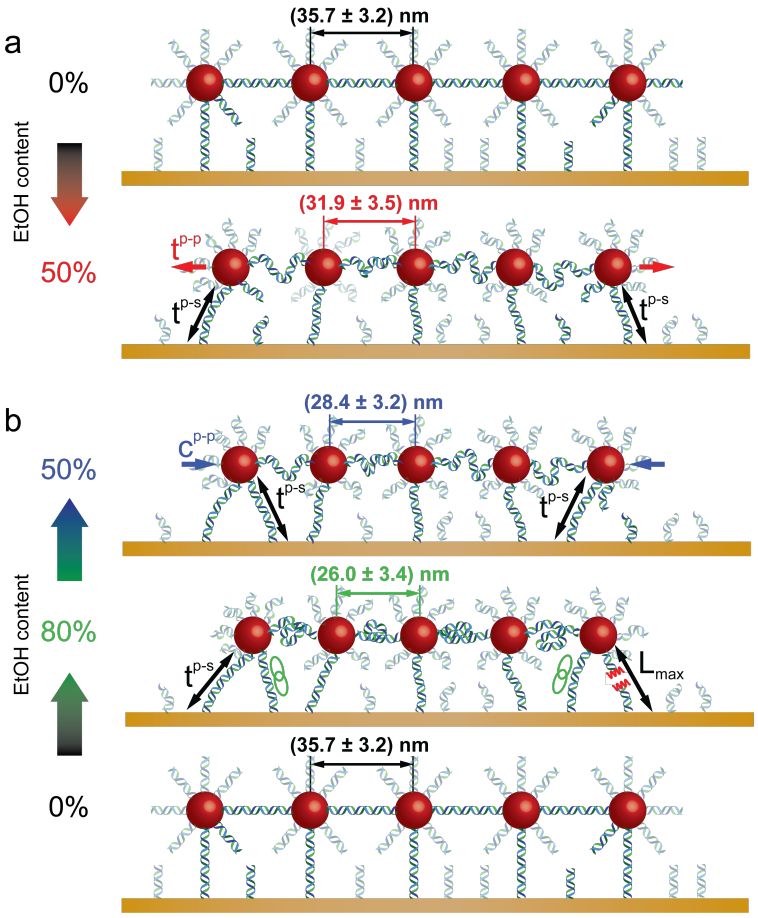

Figure 17. Reconfiguration hysteresis of substrate-linked 2D nanoparticle superlattices due to statistical DNA bond formation/re-formation and resulting in-plane stresses on the assembly. (a) Lattice contraction in the transition 0% → 50% ethanol. Tensile stress in the particle-substrate DNA bonds (t^{p-s}) induces a net tension within the superlattice plane, which counteracts the lattice contraction. **(b)** Lattice contraction upon 0% → 80% → 50% adjustment of the ethanol concentration. The substantial (> 60%) contraction of the particle-particle DNA bonds at 80% ethanol content creates the possibility for the statistical formation of new particle-substrate DNA bonds and may induce "bond breaking" (melting of all hybridized bases) if the stretching exceeds the maximum elastic length L_{max}. The newly formed linkages and the resulting in-plane compression again counteract the expansion of the superlattice upon lowering of the ethanol concentration to 50%.

Reference: M. Shekhirev, E. Sutter, and P. Sutter, "*In Situ Atomic Force Microscopy of the Reconfiguration of On-Surface Self-Assembled DNA-Nanoparticle Superlattices*", Advanced Functional Materials **29**, 1806924 (2019).

Products:

A. Publications:

Reference (1): P. Sutter, B. Zhang, and E. Sutter, "Radiation damage during in situ electron microscopy of DNA-mediated nanoparticle assemblies in solution", Nanoscale **10**, 12674 (2018) [DOI: 10.1039/C8NR04087G]

Reference (2): E. Sutter, B. Zhang, and P. Sutter, "In-Situ Electron Microscopy of the Self-Assembly of Single-Stranded DNA-Functionalized Au Nanoparticles in Aqueous Solution", Nanoscale **11**, 34 (2019). [DOI: 10.1039/C8NR08421A]

Reference (3): M. Shekirev, E. Sutter and P. Sutter, "In-Situ Atomic Force Microscopy of the Reconfiguration of On-Surface Self-Assembled DNA-Nanoparticle Superlattices", Advanced Functional Materials **29,** 1806924 (2019). [DOI: 10.1002/adfm.201806924]

Reference (4): E. Sutter, "Colloidal Nanostructures: In-Situ Electron Microscopy of Plasmon-Mediated Synthesis, Chemistry and Self-Assembly", Microscopy and Microanalysis **25**, Suppl. S2, 1404 (2019). [DOI: 10.1017/S143192761900775X]

Reference (5): M. Sun, Y. Li, B. Zhang, C. Argyropoulos, P. Sutter, and E. Sutter, "Plasmonic Effects on the Growth of Ag Nanocrystals in Solutions", Langmuir **36**, 2044 (2020). [DOI: 10. 1021/acs.langmuir.9b03765]

Reference (6): E. Sutter, B. Zhang and P. Sutter, "DNA-Mediated Three-Dimensional Assembly of Hollow Au-Ag Alloy Nanocages as Plasmonic Crystals", ACS Appl. Nano Mater. **3,** 8068 (2020). [DOI: 10.1021/acsanm.0c01528]

ACS Appl. Nano Mater. Cover [https://pubs.acs.org/toc/aanmf6/3/8]

Reference (7): E. Sutter, B. Zhang, and P. Sutter, "Single-Strand DNA-Nanorod Conjugates - Tunable Anisotropic Colloids for On-Demand Self-Assembly", J. of Colloid and Interface Science **586**, 847 (2021).[DOI:10.1016/j.jcis.2020.11.009]

Reference (8): (Invited Article) P. Sutter, and E. Sutter, "Real-Time Electron Microscopy of Nanocrystal Synthesis, Transformations, and Self-Assembly in Solution", Accounts of Chem. Research, special issue on "Transformative Inorganic Nanocrystals", **54,** 11 (2021). [DOI: 10.1021/acs.accounts.0c00678]

B. Invited Talks

(1) Eli Sutter, "Colloidal Nano-structures: In-Situ Electron Microscopy of Plasmon-Mediated Synthesis, Chemistry and Self-Assembly", 21st American Conference on Crystal Growth and Epitaxy (ACCGE-21), Santa Fe (NM), August 1, 2017 (Invited).

(2) Eli Sutter, "In-situ Electron Microscopy of Synthesis, Chemistry and Self-Assembly of Colloidal Nanostructures", International Workshop on Liquid Phase Electron Microscopy, Eindhoven (Netherlands), September 19, 2017 (Invited).

(3) Eli Sutter, "In-situ Electron Microscopy of Plasmon Mediated Synthesis, Chemistry and Self-Assembly of Colloidal Nanostructures", Pharmaceutical Sciences, University of Nebraska Medical Center, January 19, 2018 (Invited).

(4) Eli Sutter, "Design of New Materials in Solution: Insights from In-Situ Electron Microscopy", International Conference on Low Dimensional Quantum Materials, Snowbird (UT), March 13, 2018 (Invited).

(5) Peter Sutter, "Real-Time Microscopy of Electron-Beam Induced Transformations: From 2D Materials to Anisotropic Nano-Colloids", 2017 MRS Spring Meeting, Symposium MA03—Directed Matter—Atom-by-Atom Assembly with Electron Beams and Scanning Probes, Phoenix (AZ), April 3, 2018 (Invited).
(6) Eli Sutter, "Design of New Materials in Solution: Insights from In-Situ Electron Microscopy", International Materials Research Congress, Cancun (Mexico), August 20, 2018 (Invited).
(7) E. Sutter, "Real-Time Microscopy of Electron-Beam Induced Transformations: From 2D Materials to Anisotropic Nano-Colloids", International Nanotechnology Conference, Vienna, Austria, September 3-5, 2018 (Invited).
(8) E. Sutter, "Design of New Materials: Insights from In-Situ Electron Microscopy", University of Nebraska-Lincoln Conference for Undergraduate Women in Physical Sciences (WoPhyS '18), October 12, 2018 (Keynote).
(9) E. Sutter, "Self-Assembly of Nanocrystals in Solution: Insights from In-Situ Electron Microscopy"**,** MRS Spring Meeting, Phoenix (AZ), April 24, 2019 (Invited).
(10) E. Sutter, "Colloidal Nanostructures: In-Situ Electron Microscopy of Plasmon-Mediated Synthesis, Chemistry and Self-Assembly"**,** Microscopy and Microanalysis Meeting, Portland OR, August 6, 2019 (Invited).
(11) M. Shekirev, E. Sutter, P. Sutter, "In-Situ Atomic Force Microscopy of DNA-Mediated Nanoparticle Assemblies in Solution", MRS Fall Meeting, Boston (MA), November 26, 2018.
(12) E. Sutter, "Designing New Materials: In-Situ Imaging, Measurements, and Manipulation in the Electron Microscope", University of Notre Dame, South Bend (IN), November 5, 2019. (Invited)