STRUCTURAL BIOLOGY

# Peering inside protein complexes with AFM

**Atomic force microscopy is applied to image the location of chemical groups inside single protein complexes.**

Atomic force microscopy (AFM) is a versatile tool that, among many applications, allows researchers to image single molecules, all the way down to the atomic level. This technique produces a topographic map of molecular structure. However, imaging details of the internal structure of a large molecule such as a protein complex is typically not possible with AFM.

In recent work, Ozgur Sahin of Columbia University and his postdoc Duckhoe Kim developed a method that can be applied to peer inside a protein complex by targeting specific chemical groups and tugging on them with an AFM cantilever. They created short, single-stranded DNA probes that are functionalized with a binding moiety on one end. These DNA probes are designed to hybridize with complementary DNA probes tethered to an AFM cantilever. Sahin and Kim designed this cantilever-tethered probe sequence to contain two different regions that hybridize to two different complementary DNA molecules used as the binding probes. By doing this, they could use the different AFM force-time waveforms generated as a result of hybridization to one complementary sequence or another to distinguish distinct binding interactions.

Sahin and Kim initially experimented with the cantilever-tethered probe sequence by testing its interaction with two different complementary DNA probe molecules attached to a surface. "When the AFM detects an interaction, the computer highlights the corresponding pixel in the image with a specific color," explains Sahin. "We noticed that the highlighted pixels were clustered into regions less than 2 nanometers and more typically less than 1 nanometer." A careful statistical analysis of the data revealed that their AFM setup had subnanometer resolution. Because they achieved such high resolution, the researchers were encouraged to apply the approach to locate specific chemical moieties within a protein complex.

SENSORS AND PROBES

## SITE-SPECIFIC RNA LABELING IN MAMMALIAN CELLS

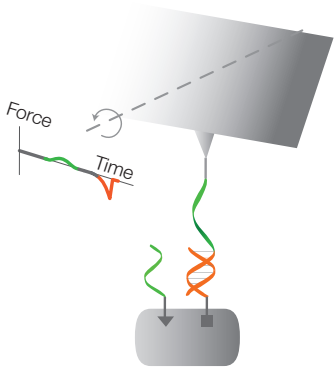
**A new method uses click chemistry to covalently modify any RNA of interest.**

RNAs have crucial roles in diverse cellular processes, and studying these RNAs in cells is important for revealing their functions. However, methods for site-specific, covalent tagging of RNAs have lagged behind those for proteins. For these reasons, Jiangyun Wang at Beijing National Laboratory for Molecular Sciences and a team of researchers worked to develop a new method for covalent modification of any RNA of interest in mammalian cells.

The method employs an archaeal tRNA synthetase (Tias) that modifies its cognate tRNA ($tRNA^{Ile2}$) by adding a molecule called agmatine. The authors found that Tias could also be used to covalently add agmatine analogs that contain a click-chemistry handle. To label an RNA of interest, researchers first genetically tag it by incorporating the $tRNA^{Ile2}$ sequence into its gene. Then this tagged gene is transcribed in cells that contain Tias and the agmatine analog. Tias then specifically adds the agmatine analog to the tagged RNA. Finally, click chemistry can be used to add functional groups to the RNA for a range of applications from fluorescence imaging to nuclear magnetic resonance spectroscopy.

According to Wang, this work was inspired by the team's previous research on the mechanism of recognition between codons in mRNA and anticodons in tRNA. In its normal cellular context, chemical modification by Tias ensures that $tRNA^{Ile2}$ decodes the correct codon. Wang says that he and his colleagues were "fascinated by the chemical basis for this novel codon-anticodon interaction and decided to investigate further," which ultimately led to the development of this new labeling strategy.

The work led to many new insights regarding the structure-function relationship of Tias, as the team solved the crystal structure of Tias in complex with one of the




Chemically specific imaging using complementary DNA labels and AFM. Figure reproduced from Kim and Sahin, Nature Publishing Group.

They put the method to the test with the well-studied streptavidin-biotin complex. They made two biotinylated DNA probes with distinct sequences and designed a cantilever-tethered DNA probe with two regions complementary to the two biotinylated probes. By moving the AFM cantilever over streptavidin complexes on a surface, they could detect locations where biotinylated DNA probes were present. After using some straightforward geometry to calculate distances between biotin-binding sites in a single streptavidin complex, they found that the measured locations of the sites were within 2 angstroms of their locations in the known crystal structure, indicating good agreement.

Sahin notes that because many proteins and complexes are difficult to crystallize, the method could have complementary value to traditional structure determination tools. He believes that the method will be readily adaptable to problems such as studying interactions between DNA or RNA and proteins. With further development, the approach could also be applied to make measurements of distances between amino acids, which in principle could be used to determine protein structure. "I anticipate that chemically linking DNA labels to specific amino acids would be the primary challenge in doing this," says Sahin.

However, Sahin is more excited about the possibilities of this single-molecule approach in studying protein motions, something that is particularly difficult using crystallography. "The fact that our method works in physiologically relevant conditions makes it an attractive option to probe dynamics of molecules," he says.

**Allison Doerr**

**RESEARCH PAPERS**

Kim, D. & Sahin, O. Imaging and three-dimensional reconstruction of chemical groups inside a protein complex using atomic force microscopy. *Nat. Nanotechnol.* **10**, 264–269 (2015).

agmatine analogs. For example, they learned that Tias likely has a flexible binding pocket because it can bind agmatine derivatives of different sizes. Wang recalls that they were also surprised to learn "that Tias-mediated chemical modification and labeling can tolerate any 5′ and 3′ extensions on $tRNA^{Ile2}$." Wang considers this versatility "truly amazing" and says it "laid foundation for using our technology to achieve RNA labeling and imaging in mammalian cells."

To demonstrate that this was feasible, they first tested whether Tias could add the agmatine analog directly to $tRNA^{Ile2}$ and whether these tRNAs could then be labeled with fluorescent dyes using click chemistry. Here, they observed labeling that was dependent upon the addition of the agmatine analog. Ultimately, they moved into mammalian cells, where they labeled 5S, an abundant ribosomal RNA, with $tRNA^{Ile2}$ and showed that 5S could be imaged after the $tRNA^{Ile2}$ was modified by addition of dye.

Future work is underway to reach an important goal: making this labeling strategy compatible with live-cell imaging. In this case, the challenge is to develop cell-permeable dyes for click chemistry with the agmatine analogs.

In addition to this work, Wang and his team are interested in using this method to study the biological roles of RNAs. Wang notes that "there are tens of thousands of RNA species in mammalian cells, and it has been realized that their localizations are essential for their proper functions in cells." He says he and his team are "collaborating with experts in super-resolution imaging and noncoding RNA research and are currently using our new technology for noncoding RNA imaging."

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Li, F. *et al.* A covalent approach for site-specific RNA labeling in mammalian cells. *Angew. Chem. Int. Ed. Engl.* doi:10.1002/anie.201410433 (18 February 2015).