

A really close look at molecules

(By Johan Svenson, scientist, University of Tromsø)

With a “magnifying glass”, a highly sophisticated atomic force microscope (AFM) scientists have recently developed a technique that allows single molecule visualization with atomic resolution.

Solving the structure of an unknown compound used to be a laborious task before modern spectroscopic methods such as nuclear magnetic resonance (NMR), mass spectrometry (MS) and X-ray crystallography (X-ray) were developed into what they are today. Scientists no longer dwell over functional group specific derivatization reactions and the good old trusted Bunsen burner is generally safely stowed away.

These days, modern spectroscopy used in conjunction with powerful computers generally allow for a rapid and straightforward determination of the bond connectivity and overall three-dimensional structure of any novel compound found. Provided that the compound is available in sufficient amounts, there is little preventing a rapid structural establishment.

But what happens once the interesting compound is not available in high enough amounts to generate crystals for X-ray analysis and display a chemical composition that leave gaps in the NMR data? If you asked a kid to solve this issue they would most likely suggest that the panacea to the problem with low amounts and challenging molecular compositions would be something resembling a big enough “magnifying glass” which allowed the curious to have a look at what the molecule actually looked like at the atomic level. You don’t need a Ph.D. to realize that such a gadget would come in quite handy but surely, such a tool is science fiction or wishful thinking isn’t it?

Actually it isn’t anymore. Scientists at IBM in Zürich, led by Dr. Gerhard Meyer and Dr. Leo Gross are performing these studies as we speak and their initial results are quite astonishing. Their “magnifying glass” is a highly sophisticated atomic force microscope (AFM) and they have recently developed a technique that allows single molecule visualization with atomic resolution.

AFM is a technique widely employed to study matter at the nanoscale. The technique is an advancement of the scanning tunneling microscope (STM) developed by IBM which awarded the inventors the Nobel Prize in physics in 1986. Several versions of AFM exist but the underlying principles are shared. A cantilever terminated with a fine tip is slowly moved across the surface studied, either in contact with the surface or maintained at a constant distance. A range of interactions between the tip of the cantilever and the surface generates measurable signals that can be translated into an accurate, highly resolved image of the surface and can also provide information about the elemental composition.

While significant progress within the microscopy field has been experienced during the last years, the atomic resolution of a single molecule was only recently achieved. Findings from the STM field suggest that modification of the tip apex by atomic manipulation can have profound effect on the image resolution. This was explored also for AFM and it was shown that by modifying the tip on the atomic level, significant improvement in the contrast could be achieved. The key development was the discovery that adsorbing a molecule of CO onto the tip (illustrated in Figure 1.) generated spectacular enhancement of the atomic scale contrast.

In the first “proof-of principle” experiment, the CO-terminated tip was moved at a constant, optimized distance (~ 3.5 Å) over the substrate, pentacene on Cu(III) generating remarkably clear images of the individual molecules as shown in Figure 2.

The energies involved in the interactions between the modified tip and the surface were calculated using density functional theory (DFT) and compared to experimentally measured forces. It was shown that the increased contrast actually arises from repulsive forces as opposed to attrac-

tive forces such as electrostatic interaction and Van der Waals forces suggesting that a close proximity to the surface is needed to perform these experiments.

The constant pursuit for new drugs to treat a range of diseases has forced the scientists to sample the most extreme environments on the planet in search of sturdy organisms capable of producing compounds suited for drug development. While several interesting and promising drug leads have been isolated from a range of extremophilic organisms, these compounds generally have one thing in common: They are only available in very small amounts. Chemical synthesis is therefore needed to provide sufficient material for further studies and development. Synthesis is however not possible without a structure and the structure determination is thus an early bottleneck. For example, the approved antitumoral compound ecteinascidin (Trabectedin/Yondelis) had its final structural elucidation delayed by two decades due to insufficient amounts which prevented essential experiments.

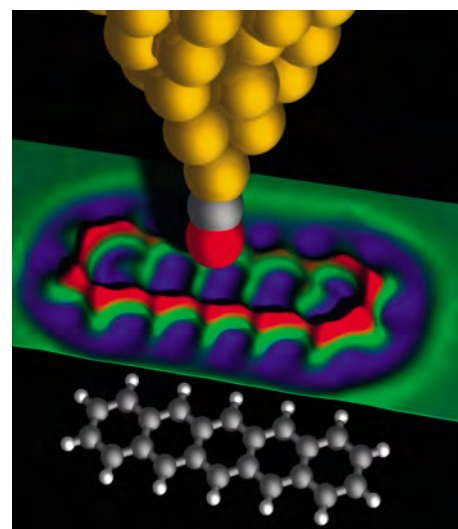


Figure 1. Schematic representation of the AFM experiment where the CO-terminated tip of the cantilever is moved in close proximity over an adsorbed pentacene molecule (grey) which generates a highly resolved image. ©IBM

Figure 2. STM and AFM images of pentacene on Cu(111). A represents a model of the compound while B is an STM image of it. C and D represent AFM images obtained with a CO-modified tip allowing for significantly increased resolution of the individual molecules. ©AAAS

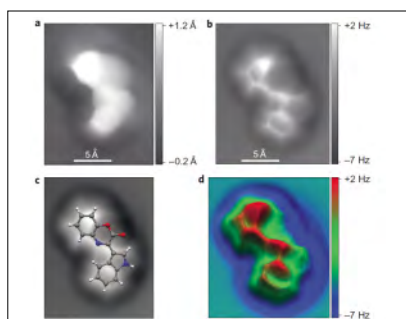
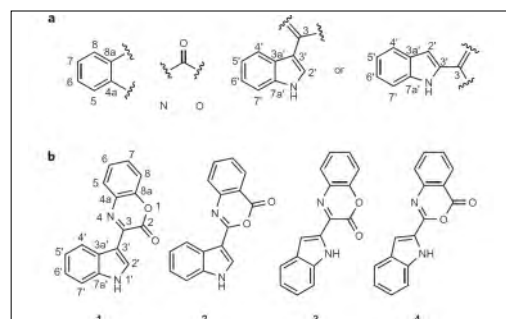
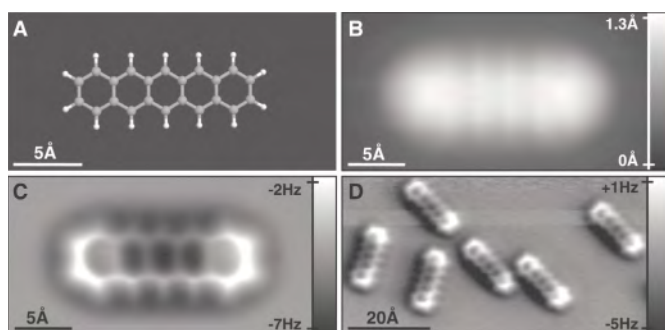


Figure 3. Left: Substructures and plausible compounds derived from the NMR data. Compound 1 is the now accepted structure of cephalandole A while 2 was the previously misassigned structure. Right: STM (a) and AFM measurements (b-d) of cephalandole A with CO-functionalized tip which helped the researchers to rule out previously plausible structures. ©Macmillan Publishers Limited

To investigate if the improved AFM technology could be employed on such compounds, Gross and coworkers teamed up with Marcel Jaspars from the Marine Biodiscovery Centre in Aberdeen. They were working on a compound isolated from a pressure tolerant bacteria, collected at the deepest place on Earth, the Mariana Trench. The compound, tentatively identified as cephalandole A, was used as model “problem molecule” which due to its low number of protons in relation to other elements generated several plausible structures shown in Figure 3 using state-of-the-art NMR analysis. A low number of protons in a molecule can prevent the generation of useful overlapping molecular fragments that allows the unambiguous assembly of a single final structure. The protons in a molecule can further be used to generate information about the carbon atoms and the inherent 100-fold lower NMR-sensitivity of carbon (due to only 1.1% being the NMR active ^{13}C isotope) thus becomes even more problematic to deal with when the compound is low on protons.

AFM analysis of the compound with the CO-modified tip generated an image where the molecular structure was partly visible. Together with calculations of the adsorption geometry it was possible to dismiss three of

the four plausible structures (2-4 in Figure 3) suggested from the NMR experiment.

So far the technique had solely been used on known compounds and it was just recently that the technique was employed to solve the structure of an unknown halogenated natural product for the first time. The compounds studied were isolated by marine scientists in Tromsø from the marine coral-like hydrozoan *Thuiaria breitfussi* (Figure 4) and the scientists were once more faced with an interesting compound in minute amounts and a chemical composition with a low ratio of protons giving the NMR-people grey hair. AFM and computer aided structure elucidation was thus further employed to the problem and the structure of two isomeric compounds, breitfussin A & B could eventually be revealed.

This AFM technique has shown its worth and may be developed further into a highly useful tool for structure determination and an important complement to synthesis in addition to several other surface applications. Current limitations are mainly related to the need for a planar structure as the atomic resolution is only possible for the topmost layer of an adsorbed molecule. However many natural products are planar (about 20% of the marine ones) suggesting that the method may still be valuable in this

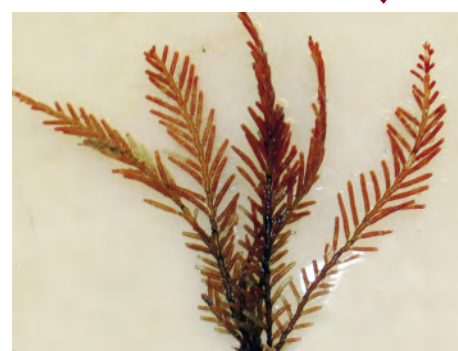


Figure 4. The hydrozoan *Thuiaria breitfussi* from which minute amounts of the breitfussins were isolated and subsequently later structurally resolved using AFM and NMR.

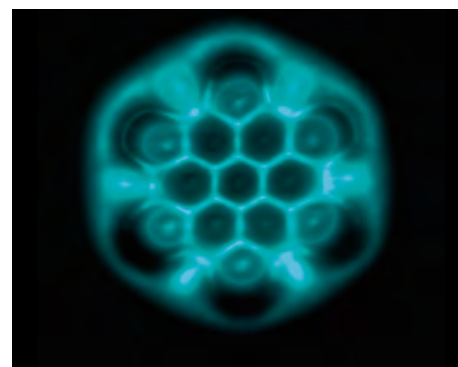


Figure 5. A stunning AFM image of hexabenzocoronene. ©AAAS

field. As for generation of amazing “atomic photage” there is currently little competition as the recent image of hexabenzocoronene shows (Figure 5.), clearly illustrating the bond lengths and angles our poor high school chemistry teachers once tried to convince us about. [KB](#)

References

- Gross, L; Mohn, F; Moll, N; Liljeroth, P; Meyer, G. The chemical structure of a molecule resolved by atomic force microscopy. *Science* **2009**, 325, 1110-1114.
- Gross, L; Mohn, F; Moll, N; Meyer, G; Ebel, R; Abdel-Mageed, WM; Jaspars, M. Organic structure determination using atomic-resolution scanning probe microscopy. *Nature Chemistry* **2010**, 2, 821-825.
- Hanssen, KO; Schuler, B; Williams, AJ; Demissie, TB; Hansen, E; Andersen, JH; Svenson, J; Blinov, K; Repisky, M; Mohn, F; Meyer, G; Svendsen, JS; Ruud, K; Elyashberg, M; Gross, L; Jaspars, M; Isaksson, J. A Combined Atomic Force Microscopy and Computational Approach for the Structural Elucidation of Breitfussin A and B: Highly Modified Halogenated Dipeptides from *Thuiaria breitfussi*. *Angewandte Chemie-international edition* **2012** 51, 12238-12241.
- Gross, L; Mohn, F; Moll, N; Schuler, B; Criado, A; Guitian, E; Pena, D; Gourdon, A; Meyer, G. Bond-order discrimination by atomic force microscopy. *Science* **2012** 337, 1326-1329.