Determining the 3D Structure of HIV-1 Protease

In his News article "When Pharma merges, R&D is the dowry" (special issue on Drug Discovery, 17 Mar., p. 1952), Bruce Agnew writes that "Merck researchers were the first to determine the three-dimensional structure of the HIV-1 protease enzyme in 1989," and Roger Perlmutter is quoted as saying, "we published that structure so that everybody else could work on it, too." However, these statements do not accurately reflect the course of events.

The human immunodeficiency virus–type 1 (HIV-1) protease structure determined crystallographically by Merck researchers using recombinantly expressed HIV-1 protease was published in *Nature* in early 1989 (*1*). This structure was of the unliganded (empty active site) enzyme and was seriously flawed because the low resolution of the data led to an incorrect tracing of the polypeptide chain at the dimer interface. In any event, only the coordinates of the carbon alpha atoms of the main chain were deposited with the Protein Data Bank (PDB). Such limited data for the unliganded enzyme were of little, if any, use to researchers undertaking structure-based drug design.

The first complete and correct structure of the HIV-1 protease was determined crystallographically at the National Cancer Institute (NCI) using enzyme prepared by total chemical synthesis in Kent's laboratory at the California Institute of Technology, and the structure was published in August 1989 (*2*). The more important structure of an HIV-1 protease–ligand complex was also determined at NCI, again using enzyme prepared by total chemical synthesis in Kent's laboratory at Caltech with a substrate-derived inhibitor prepared by Marshall's laboratory at Washington University at St. Louis. That structure was published in December 1989 (*3*). These structures of the synthetic enzyme were of high resolution and of good quality, providing an appropriate target for structure-based drug design. The full coordinates for both structures were immediately deposited in the PDB and were made freely available to researchers.

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Response

As Kent, Marshall, and Wlodawer make plain, numerous groups (including their own) made contributions to the determination of the structure of the HIV-1 protease. There appear to be no serious issues of contention between us. Merck Research Laboratories made public a structural analysis of the HIV-1 protease and deposited the data in the PDB in early 1989. The structure was not "seriously flawed," although we readily acknowledge that it was incomplete. It provided the best, and at the time the only, representation of the structure of the HIV-1 protease. Resolution of the alpha chain backbone was a fundamental first step.

Crystallographic analysis is typically iterative, and subsequent work by Kent, Marshall, and Wlodawer clearly provided substantive and more detailed information. The important point, as I indicated in Agnew's article, is that the initial publication of structural data by Navia *et al.* accelerated the development of protease inhibitors by several pharmaceutical companies, to the general benefit of patients suffering from HIV infection.

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Annotation of the Human Genome

The News article "Are sequencers ready to 'annotate' the human genome?" by Elizabeth Pennisi (special issue on the *Drosophila* Genome, 24 Mar., p. 2183) is especially timely and provocative. Pennisi mentions two ideas: a small group gathering at a centralized annotation jamboree, or a distributed, Web-based system that would allow anyone to contribute annotations with a "smart browser" that would merge all efforts. I favor the essence of the second proposal because it provides a more democratic and more "biological" approach to an all-important problem.

There is, however, a third approach for annotating the human genome (providing at least the putative start, stop, and structure of each gene) that is, in a sense, already extant: extend the capabilities of the biological science literature. The current journal system is decentralized, yet most research articles adhere to common standards that make them ideal for annotation: (i) Each article associates a bit of annotation with a distinct time and place and with specific, responsible parties. (ii) Attentive scholarly referencing and footnoting provide a way to connect bits of annotation and allow for continuous "updates." (iii) Peer review and editing provide a proven quality-control mechanism. (iv) Publication is an established indicator of scientific productivity; consequently, scientists already have an incentive to provide the information, whereas database submissions are often regarded as a chore.

The main drawback of current journal article formats is that they are not very "computer-parseable," or suitable for bulk annotation of thousands of genes. However, by adding sections of highly structured text to each article (that is, extended keywords and using a controlled vocabulary) and linking subparts of an article to relevant database identifiers, one can envision how a "literature annotation standard" could readily be interpreted by computers. Furthermore, if an article could be linked to a large "supplementary materials" data file with simple annotations for many genes (for example, lists of all the membrane proteins in the *Caenorhabditis elegans* genome), one would have a mechanism for bulk annotation. Further standardization could be achieved if the article described defined ways in which the data file might be updated over time and if the supplementary materials were refereed and evaluated with the text of the article.

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Window on the Early Earth

Carl Zimmer's News Focus article "Ancient continent opens window on the early Earth" (17 Dec., p. 2254) highlights interpretations of a very old fragment of Canadian continental crust by a team headed by Wouter Bleeker and Richard Stern. This team has substantially advanced understanding of the early geologic evolution of the Slave Province, but Zimmer attributes solely to these scientists the model of an ancient protocontinent overlain by a shallow-water sedimentary sequence in the western Slave Province, and collision of this protocontinent with a younger arc terrane 2.7 to 2.6 billion years ago. Virtually the same model was conceived 15 years before and published in reputable journals, which is nowhere mentioned in the article.

The model attributed to Bleeker and Stern stems from years of work, including more than 9 months in the field, mapping the distribution of and determining basic geological relationships between ancient basement rocks and surrounding units (*1*). The boundaries of this old crustal fragment were defined on the basis of field relationships and a limited number of uranium-lead (U-Pb) dates, and the name "Anton terrane" was proposed along with its interpretation as an ancient continent (*2*). The boundaries of the old continent have only been slightly modified on the map presented in *Science*, and most of the major conclusions regarding the history of this continent (formation at 4.0 to 2.9 billion years ago, followed by rifting at 2.8 to 2.7 billion years ago, leading to the gneiss being overlain by shallow-water sediments, including quartzite, then collision with an arc terrane at 2.7 to 2.6 billion years ago) are also the same as those presented as a new model in *Science*. Those conclusions were initially controversial when first proposed (*1, 2*), stimulating the inception of many new projects, including those reported by Zimmer. Although there is now much more quantitative and analytical data (especially U-Pb geochronology) that generally supports those early ideas, these new data have not dramatically changed the basic framework of the field geology–based models for the Slave Province, nor the known distribution of the old fragment of continental crust first published in 1986. Differences remain in the interpretation of some mafic magmatic sequences (*3*).

Zimmer quotes Stern on the use of the sensitive high-resolution ion microprobe (SHRIMP) for deciphering the geologic history of the Slave Province as "nobody would want to go digging around in a place like that without such a tool. You couldn't solve anything without it." This tool has revealed fantastic chronological details that were hitherto unknown, and pushed back the oldest known ages from the Anton terrane by nearly 100 million years. However, despite 15 years of intensive study, analysis, and debate, the basic models outlined 15 years ago based on interpretation of fundamental field relationships, structural geology, and plate tectonic principles for some of the world's oldest rocks have withstood the test of time and new technology.

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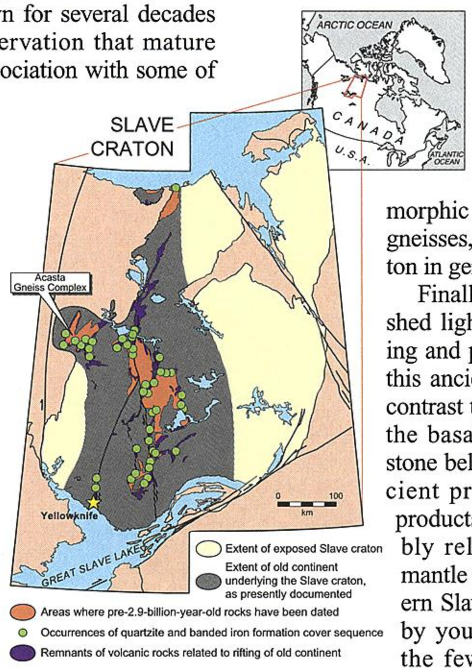
Response

Zimmer's article resulted from a visit by him to the Acasta gneisses in the company of one of us. His article describes some aspects of our work on the Acasta gneisses (*1, 2*) and how these ancient rocks fit in the context of the larger Archean Slave craton [for example, (*3, 4*)]. Our request to have references included in the article was declined by *Science*. In no way do we wish to minimize the significance of earlier work, including that of Kusky [for example, (*5, 6*)]. However, we take strong exception to most of the points raised by Kusky in his letter.

First, as acknowledged in the article, the existence of ancient gneisses in the Slave craton has been known for several decades (*7*). Second, the observation that mature quartzites occur in association with some of these gneisses was made by others, either before or simultaneous with the work of Kusky [for example, (*8*)]. Kusky's work in the mid- to late-1980s made few, if any, contributions to either of these important subjects. At several of the key localities where we subsequently documented the existence of essentially intact unconformities, complete with preserved paleoweathering horizons, and overlain by quartzites with ultramafic sills, Kusky mapped "mélanges" with "exotic" ultramafic rocks to support his contention that the overlying greenstone belts are obducted ophiolites.



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Kusky's only effort to document an autochthonous sedimentary cover below his "obducted ophiolites" described a clastic and carbonate sedimentary sequence that demonstrably unconformably overlies—not underlies, as claimed by Kusky (*6*)—the mafic greenstone succession and is about 100 million years too young to be correlated with the basal quartzite and banded iron formation sequence documented by our work (*2, 4*). Following a model-driven approach, he described this incorrectly correlated sequence in genetically loaded terms such as "wildflysch," but did not solve the basic field relationships.

The results coming out of our work are the systematic tracing and dating of this old basement and its diagnostic cover sequence throughout much of the central Slave craton, all the way to the Acasta River area and across terrane boundaries proposed by Kusky. In the process we found no evidence for his separate Sleepy Dragon terrane, or his Contwoyto accretionary prism terrane. We also significantly changed the extent and distribution of the ancient crust.

More specifically, our work places the ~4.0-billion-year-old Acasta gneisses within this regional basement complex, such that the detailed geochronological database that is emerging from the Acasta River area [for example, (*1, 3, 9*)] has bearing on the evolution of this extensive early Archean basement complex as a whole. Our comment regarding the necessity for a microprobe technology (that is, SHRIMP) in solving geochronological problems was in reference to unravelling the polymetamorphic history of the Acasta gneisses, not to the Slave craton in general.

Finally, we have started to shed light on the precise timing and processes of rifting of this ancient protocontinent. In contrast to Kusky, we interpret the basalt-dominated greenstone belts that overlie this ancient protocontinent as the products of its rifting, possibly related to arrival of a mantle plume. That the eastern Slave craton is underlain by younger crust is one of the few points that we do share with Kusky's model, although we regard the idea that it represents an exotic arc terrane as just one of several as yet insufficiently tested hypotheses.

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