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Jan Drenth (1925–2025)

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1. Early life

Jan Drenth, professor emeritus at the University of Groningen, the Netherlands, passed away on 11 February 2025. He was born in Groningen on 20 February 1925 as one of identical twins. In addition to his twin brother, Wiendelt, he had a sister who was three years older. His father was an account manager at a paperboard company.

The twin brothers were very close and did everything together. They attended the same schools, riding their bikes each morning at the same time and in the same way. Both graduated from high school in 1942 during the German occupation and went on to study chemistry at the University of Groningen. Their identical appearance sometimes led to logistical challenges during oral exams. To avoid confusion, one professor took Jan by the hand, led him to the door and then brought Wiendelt in, thus ensuring he did not examine the same Drenth twice.

However, in March 1943 the German occupiers mandated that all students sign a 'declaration of loyalty' to them. Those who refused were barred from attending classes and were required to report for forced labor in Germany. Jan and Wiendelt refused to sign this declaration. Unable to find a safe place to go into hiding, they were deported to Germany in May 1943. Along with a dozen other students, they were sent to Wittenberge, a small town on the east bank of the Elbe river, where they worked for two years in a rayon plant (Kurmärkische Zellwolle und Zellulose AG). Fortunately, the forced labor was not as harsh as they had feared. The German officer in charge of their laboratory knew a friend of Jan and Wiendelt's father in Groningen, which eased their situation.

At the end of the war, being on the east side of the Elbe, they were liberated by the Russians, while the Americans held the west bank. In the chaotic days that followed, they managed to cross the river but still needed several weeks to make their way home to Groningen. Remarkably, despite the hardships that he endured, Jan never harbored any resentment toward Germany or Germans.

They resumed their chemistry studies, and Jan obtained his PhD in 1957 at the University of Groningen with Eelco Wiebenga as his advisor. Jan's PhD research focused on the X-ray study of crystals of enormous seed proteins (300–350 kDa). Subsequently, he spent a postdoctoral year in Barbara Low's laboratory at Columbia University, New York, working on a much smaller protein, insulin. On the boat to New York he met his future wife, Wil Nordemann.

2. Papain

After returning from his postdoctoral year, Jan was appointed assistant professor at the University of Groningen and chose to work on another plant protein: the protease papain, extracted from the latex of the papaya tree. Papain had been extensively studied biochemically by Emil Smith in the United States, and before heading home after his postdoctoral studies, Jan visited Smith, who had just completed the amino-acid sequence determination of papain. This made papain an ideal candidate for X-ray structure determination, a project that Jan undertook in Groningen together with Hans Jansonius.

Precession cameras were used to collect the diffraction intensities. In 1959 the unit cell and the first heavy-atom derivative were published, followed three years later by three additional derivatives and two planar projections of the papain crystal structure at 5 Å resolution. Another five years later a three-dimensional Fourier could be calculated at 4.5 Å resolution.

Jan was known for his skill in tinkering with instruments, which led to the design and construction of his automatic densitometer (Drenth *et al.*, 1965). This machine converted the blackness of the diffraction spots on precession films taken from protein crystals into holes in paper tape, which the university computer then converted to the integrated density of each spot on the film. Scanning a single film took many hours, and for years the characteristic sound of the scanner echoed through the corridors of the Laboratorium voor Struktuurchemie in Groningen. The instrument proved to be essential for solving the structure of papain.

Fittingly, those climbing the stairs to the laboratory in those years were greeted by an impressive 2 × 2 m print of a papain precession X-ray image on the wall. This striking display conveyed the remarkable fact that the varying blackness of the diffraction spots encoded the secret of a protein's structure: the precise mutual positions of its thousands of atoms.

Next to this image hung an equally large print depicting dozens of *Helix pomatia* hemocyanin molecules, a massive, barrel-shaped oxygen-transport protein studied by electron microscopy in the group of Jan's colleague Erni van Bruggen. Together, these images beautifully illustrated the foundation of the pioneering and successful Groningen venture into what is now known as structural biology. In later years, the research environment expanded further with the addition of groups specializing in molecular-dynamics simulations (led by Herman Berendsen) and protein NMR (led by George Robillard and Rob Kaptein), creating a very attractive and state-of-the-art center for structural biology research and teaching.

Thanks to the dedication of Jan's graduate students, Hans Jansonius and Roelof Koekoek, the three-dimensional structure of papain was fully resolved in 1968 using the multiple isomorphous replacement method, supplemented with anomalous diffraction differences from three heavy-atom derivatives (Drenth *et al.*, 1968). A remarkable feature of this structure determination was its significant contribution to correcting the amino-acid sequence of papain determined by the group of Emil Smith in the United States. The electron-density map of papain was so clear that a pseudo-amino-acid sequence could be derived from it, allowing researchers to align peptide fragments of papain with known sequences from Smith's group. A 13-amino-acid segment was missing from the previously determined amino-acid sequence, and two pieces of the sequence had been incorrectly connected. At the time, establishing the correct order of such peptide fragments was often one of the most time-consuming steps in chemical sequence determination.

Papain adopted a novel fold, and its Kendrew–Watson wire model was proudly displayed in the laboratory's corridors. The active site was found to contain an essential cysteine residue, positioned near a histidine and an asparagine, forming a so-called Cys–His–Asn triad. Remarkably, this plant protein shared key active-site features with mammalian chymotrypsin-like serine proteases. Despite having an entirely different fold, its active site had evolved into a similar functional arrangement, an example of what one might call local convergent evolution.

Papain was the fifth three-dimensional enzyme structure to be determined worldwide and the first to be elucidated in continental Europe (Rossmann, 2012). Jan Drenth's scientific reputation increased rapidly, and he traveled extensively to present the fold of papain and discuss the mode of action of this plant protease with research groups around the world. Jan liked to use a stereo projector by which stereo slides could be projected with polarized light onto a special screen. The

audience had to use special glasses to see the image in three dimensions. He often used this heavy setup for presentations in the Netherlands.

One of these lectures was given in Eindhoven, where an undergraduate, who would later become Jan's fourth graduate student, a long-standing colleague and one of the authors of this obituary (WGJH), asked why anyone would be interested in the structure of a plant protein when there are so many pressing medical problems to solve. Jan replied that it was because papain had crystallized, and its structure would offer new insights. Moreover, biology is full of surprises. This point turned out to be entirely correct. For instance, the coronavirus contains an important papain-like protease which is currently the subject of structure-based drug-design studies (Jadhav *et al.*, 2024).

3. Subtilisin

That graduate student from Eindhoven continued the successes of Jan Drenth's group by solving the structure of subtilisin, a serine protease secreted by certain bacteria. The enzyme crystallized from 50% acetone, an unusual reagent for protein crystallization (Drenth *et al.*, 1972*b*). Its structure closely resembled that of subtilisin solved using crystals obtained from high concentrations of ammonium sulfate by Christine Wright in San Diego. This finding demonstrated that, at least in many cases, the X-ray structure of a protein remains largely unaffected by the crystallization conditions (Drenth *et al.*, 1972*a*).

Like chymotrypsin, subtilisin contains a catalytic Ser–His–Asp triad, yet the two enzymes adopt entirely different folds. Subtilisin serves as another example of how enzymes from different evolutionary lineages can develop similar catalytic mechanisms while maintaining distinct structural frameworks: an instance of convergent evolution. Similar proteases have been identified in other organisms, including the *Plasmodium* parasite responsible for malaria (Mishra *et al.*, 2019).

4. Awards and collaborations

In 1969, Jan was appointed full professor, a position that he held until his retirement in 1990. In 1973, he became a member of the Royal Netherlands Academy of Arts and Sciences. In 1980, he was awarded the Royal Shell Prize for Chemistry and was elected as a member of the European Molecular Biology Organization (EMBO). He served as vice-chair of the Council of the European Molecular Biology Laboratory (EMBL) from 1985 to 1987 and as chair from 1988 to 1990.

As a professor, Jan initiated several new research projects in collaboration with biochemistry groups across the Netherlands, particularly with the biochemistry departments of Wageningen University (PHBH) and Utrecht University (PLA_2).

5. *p*-Hydroxybenzoate hydroxylase (PHBH)

The collaboration with Wageningen University involved Professor Franz Müller, who studied the enzymatic properties of various flavin-dependent enzymes, including the FAD-dependent enzyme *p*-hydroxybenzoate hydroxylase (PHBH). Structural studies of this enzyme posed a significant challenge due to its size: approximately 400 residues. Although the yellow crystals diffracted well, new hardware and software methods had to be implemented for data collection and processing.

Jan had created the ideal research environment for such a project, being well integrated into the small international protein crystallography community and supported by excellent technical staff. The first model of this enzyme was built in 1978 as a physical model (1 Å = 2 cm) using metal-frame models of amino acids, FAD and substrate in a specially made Richards box, as the existing unit was too small for this structure. This Richards box featured a vertical mirror measuring 150 × 180 cm.

Eventually, a model was also built using the research group's first interactive computer-graphics unit, with Jan, always eager to pioneer new methods, enthusiastically participating in the computer-graphics-guided model building of PHBH. The crystal structure, determined at 2.5 Å resolution, provided one of the earliest structural insights into an FAD-dependent enzyme. Subsequent studies further elucidated the mechanism of PHBH and other FAD-dependent enzymes (Wierenga *et al.*, 1979; Schierbeek *et al.*, 1989).

6. Phospholipase A_2 (PLA_2)

Jan Drenth also collaborated successfully with Gerard de Haas and Arend Slotboom (Utrecht University) on pancreatic phospholipase A_2 (PLA_2), a lipolytic enzyme that catalyzes the hydrolysis of the ester bond at the C2 position of 3-phosphoglycerides. In addition to its digestive function, PLA_2 enzymes play key roles in various physiologically important processes, including inflammation, blood platelet aggregation and acute hypersensitivity.

Jan initially worked on the porcine pancreatic enzyme, but since the bovine enzyme produced much better-diffracting crystals, the focus shifted to this variant. The crystal structure of bovine phospholipase A_2 revealed a catalytic site containing the key catalytic residue His48 and a calcium ion, both of which are essential for activity. His48 forms a charge-relay system with Asp99 and activates a water molecule that hydrolyzes the sn-2 ester bond of the phospholipid substrate (Dijkstra *et al.*, 1981). The calcium ion binds to the phosphate group of the phospholipid and stabilizes the tetrahedral transition state of the hydrolysis reaction. When bound in the active site, the phospholipid substrate retains its conformation as found in micelles and phospholipid bilayers, enabling hydrolysis without requiring structural changes of the substrate (Thunnissen *et al.*, 1990).

Next, the molecular basis of the interaction of the enzyme with micelles was investigated. Pancreatic phospholipase A_2 is synthesized as a zymogen, which is activated in the intestine by the removal of seven N-terminal residues. While the zymogen and active enzyme exhibit similar catalytic properties on monomeric substrates, the active enzyme is 100- to 1000-fold more active on aggregated substrates such as micelles.

X-ray crystallographic studies of the zymogen (Dijkstra *et al.*, 1982) and an N-terminally modified phospholipase A_2 (Dijkstra *et al.*, 1984) revealed that about 10% of the amino-acid residues, including the N-terminal region, are disordered in the zymogen and modified protein compared with the fully active enzyme. In the active enzyme, the free N-terminal α-NH_3^+ group is fully hydrogen-bonded and deeply buried within the interior of the enzyme, rigidifying the previously disordered regions. These regions include several residues known to interact with micelles, explaining why the zymogen and N-terminally modified PLA_2 have reduced affinity for aggregated phospholipids (Dijkstra *et al.*, 1984).

The integration of protein crystallography, biochemical analysis and enzymological studies made this research particularly compelling, attracting significant interest from both academic researchers and the pharmaceutical industry.

7. Later projects

After retiring, Jan Drenth embarked on two new major projects: writing the textbook *Principles of Protein X-ray Crystallography* and exploring the physical principles underlying protein crystallization.

8. *Principles of Protein X-ray Crystallography* textbook

Jan enjoyed teaching and mentoring students. Even after his official retirement in 1990, he continued lecturing on protein crystallography while simultaneously writing his textbook. This work provided detailed insights into both the theory and practice of crystallography, from crystal growth and data analysis to the intricate methods used to recover the phase information that is lost in diffraction experiments. First published in 1994, *Principles of Protein X-ray Crystallography* quickly became a standard textbook in the field, even earning a Japanese translation. Its success motivated Jan to refine and expand the content, leading to a second edition in 1999 and a third in 2007 (Drenth, 2007). After this, he shifted his focus to new interests outside science.

9. Principles of protein crystallization

While Jan's primary research focused on solving protein crystal structures to answer key questions about protein activity and function, he became increasingly fascinated by the fundamental process of protein crystallization itself, a crucial step in obtaining high-resolution structural data. At the time, theoretical understanding of crystallization lagged behind experimental advancements, leaving significant questions unanswered: How do proteins nucleate? What governs the transformation from tiny crystal nuclei to large, usable crystals?

To tackle these challenges, Jan collaborated with Cor Haas, an emeritus professor of solid-state chemistry. Together, they developed a more quantitative approach to protein crystallization, aiming to predict optimal conditions for protein crystallization in a way that would be useful for protein X-ray crystallographers. The work, funded by the University of Groningen, the European Space Organization and the European Union, resulted in more than ten publications between 1990 and 2007. Among their major findings was the critical role of density fluctuations in initiating nucleation (Drenth, 2005); the discovery, using NMR, of a measurable induction period before the first nuclei appear in solution, which is highly dependent on supersaturation levels (Drenth *et al.*, 2003; Drenth & Haas, 1998); and the theoretical explanation of the relationship between protein solubility in aqueous solutions and the second virial coefficient (Haas *et al.*, 1999). These achievements were widely recognized and extensively cited.

10. Jan Drenth: the person

Throughout his tenure at the University of Groningen, Jan and his colleagues cultivated a highly stimulating and collaborative research environment that attracted students and postdocs from around the world. His patient and expert guidance was instrumental in helping researchers bring their challenging projects to fruition. Through his research, teaching and influential textbook, Jan Drenth left a lasting mark on the field of protein crystallography, advancing both theoretical understanding and experimental methodology.

Beyond the laboratory he greatly enjoyed the annual group outings, especially those involving cycling, an activity that allowed him to indulge in one of his favorite pastimes: fixing flat tires, which inevitably occurred.

A gifted speaker, Jan was equally engaging at formal events and the lively celebrations that followed each PhD defense. The informal gatherings he and his wife, Wil, hosted, often in honor of international visitors, were also memorable occasions. Sadly, Wil passed away in 1984. Several years later, Jan met Els Baarspul, and they married in 1990, shortly before Jan's retirement.

Jan is survived by his wife Els and his son Erik from his first marriage.

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