

The Enzyme that Doesn't Act Like One

NUDT5 Controls the Production of DNA Building Blocks Through Structure, Not Catalysis

A surprising discovery reveals how a single protein helps cells decide when to make the building blocks of DNA. Researchers at CeMM, together with collaborators from the University of Oxford, have discovered that the enzyme NUDT5 acts not through its chemical activity, but as a physical “scaffold” that helps switch off a key metabolic pathway when purine levels are high. The study, published in *Science* (DOI 10.1126/science.adv4257), reveals a mechanism with implications for cancer treatment and inherited metabolic disorders.

(Vienna, 6 November 2025) Inside every cell, a finely tuned metabolic network determines when to build, recycle, or stop producing essential molecules. A central part of this network is folate metabolism, a process that provides vital chemical units for the synthesis of DNA, RNA, and amino acids. When this system is disturbed - for example through genetic mutations or a lack of dietary folates - the consequences can range from developmental disorders to cancer.

Now, researchers from CeMM, the Research Center for Molecular Medicine of the Austrian Academy of Sciences, together with collaborators from the University of Oxford, have identified an unexpected player in this metabolic balance: the enzyme NUDT5. Their study, published in *Science*, shows that NUDT5 helps to switch off purine production - the chemical pathway that generates the building blocks of DNA - but does so without using its enzymatic activity. Instead, the protein acts as a kind of molecular scaffold that physically restrains a key biosynthetic step when purine levels are already high.

A new role for an old enzyme

Purines are essential molecules that cells use to build DNA and RNA and to store energy. They can be recycled from existing material, or produced from scratch through the so-called *de novo* pathway - an energy-intensive process that must be tightly controlled.

In their study, the researchers explored this control mechanism by studying cells with mutations in the gene MTHFD1, a crucial enzyme in the folate cycle. Folate metabolism provides the one-carbon units required for purine synthesis, and defects in this pathway cause rare genetic diseases and influence cancer risk.

Using a combination of genetic screening, metabolomics, and chemical biology, the team discovered that the protein NUDT5 interacts with another enzyme, PPAT, which catalyzes the first step of purine synthesis. When purine levels rise, NUDT5 binds to PPAT and likely locks it into an inactive form - effectively telling the cell to stop producing more purines.

Surprisingly, this function of NUDT5 does not rely on its known enzymatic activity, which breaks down nucleotide derivatives. Even when its catalytic site was chemically blocked or genetically disabled, the protein continued to regulate purine synthesis. Only when NUDT5 was completely removed - either through genetic knockout or a newly developed molecule that selectively degrades it - did cells lose this control mechanism.

Metabolic control with medical implications

The discovery sheds new light on how cells sense and respond to changes in their metabolic environment. “NUDT5 has long been classified as an enzyme that hydrolyzes metabolites,” says Stefan Kubicek, Principal Investigator at CeMM and senior author of the study. “But our work reveals a completely different role - it acts as a structural regulator that determines whether the cell keeps producing purines or not.”

This mechanism may also explain why some cells become resistant to certain cancer drugs. “Many chemotherapies, such as 6-thioguanine, work by mimicking purine molecules and blocking DNA synthesis”, explains Tuan-Anh Nguyen, co-first author of the study. “But we found that cells without functional NUDT5–PPAT interaction were less sensitive to these treatments, suggesting that mutations in NUDT5 could contribute to drug resistance in tumors.” The key role of NUDT5 in controlling cancer drug sensitivity is also supported by similar findings from Ralph DeBerardinis’ laboratory that are also published in the same issue of *Science*.

In addition, the research connects the dots between folate metabolism, purine synthesis, and diseases caused by MTHFD1 deficiency, a rare genetic disorder that affects immune and neurological development. “Because the folate and purine pathways are tightly linked, understanding this regulatory network could eventually inform new therapeutic approaches”, Jung-Ming George Lin, co-first author of the study, adds.

The collaborators in Kilian Huber’s lab in Oxford also developed a chemical degrader called dNUDT5, which can selectively eliminate NUDT5 from cells. This tool will allow scientists to study the pathway in more detail and may offer future possibilities for protecting healthy cells from chemotherapy side effects.

“Our findings highlight that enzymes not only can act via the chemical reactions they catalyze, but also through their structure,” concludes Kubicek. “Sometimes, it’s the physical presence of a protein that makes the crucial difference.”

Pictures attached

Photo: The authors of the study Tuan-Anh Nguyen, Jung-Ming George Lin and Stefan Kubicek (f.l.t.r.) © CeMM

Graphic: Alphafold interaction prediction between NUDT5 dimers (red) and PPAT tetramer (blue) © Tuan-Anh Nguyen

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The **CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences** is an international, independent and interdisciplinary research institution for molecular medicine under the scientific direction of Giulio Superti-Furga. CeMM is oriented towards medical needs and integrates basic research and clinical expertise to develop innovative diagnostic and therapeutic approaches for precision medicine. Research focuses on cancer, inflammation, metabolic and immune disorders, rare diseases and aging. The Institute's research building is located on the campus of the Medical University and the Vienna General Hospital.
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