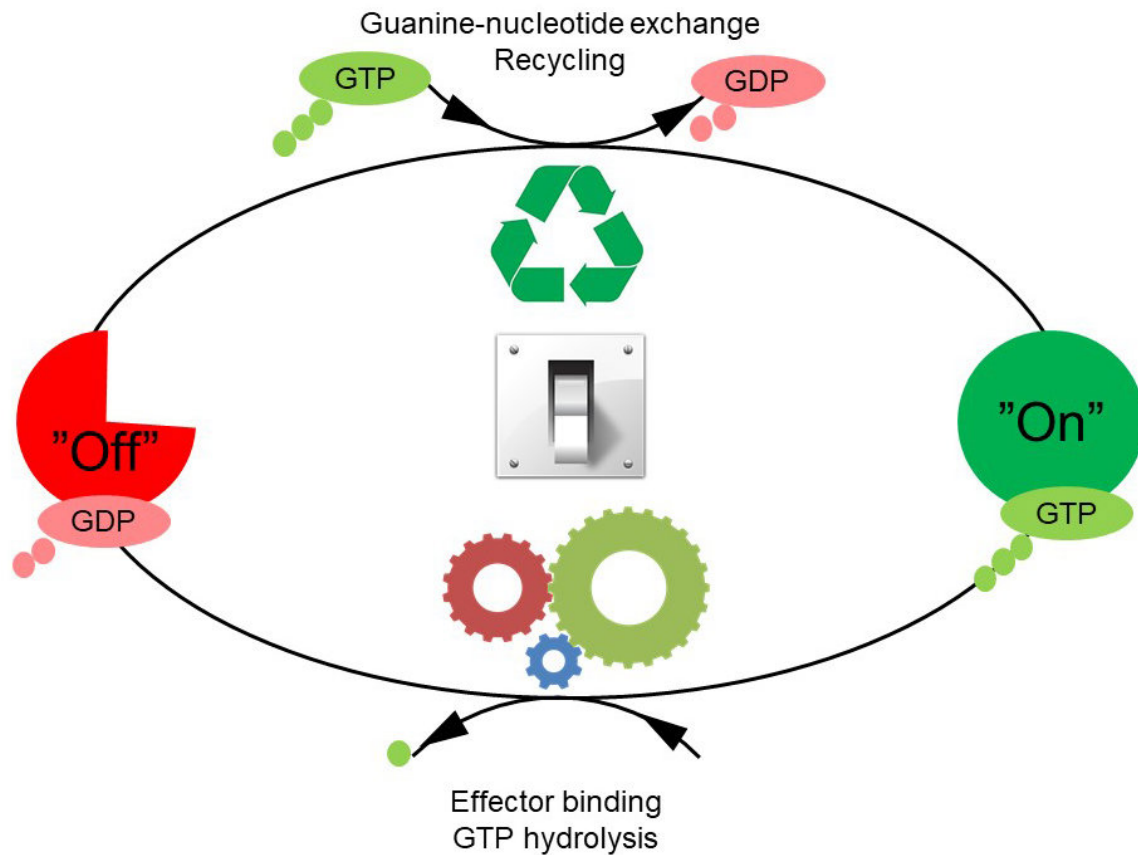


Molecular switches are not just 'on' or 'off'

September 11 2018, by Lisbeth Heilesen



When a GTPase is bound to GTP (right), it is active or 'on'. In this state, the GTPase can bind to so-called effector molecules (below), which transmit the GTPase's signal further within the cell. The interaction with the effector causes the GTPase to hydrolyse the bound GTP, thereby rendering the GTPase to its inactive "off" GDP-bound form (left). The GTPase can be reactivated via interaction with a guanine-nucleotide exchange factor that promotes the replacement of the bound GDP with GTP (top). Credit: Charlotte Rohde Knudsen

The GTPases constitute a very large protein family, whose members are involved in the control of cell growth, transport of molecules, synthesis of other proteins, etc. Despite the many functions of the GTPases, they follow a common cyclic pattern (Figure 1). The activity of the GTPases is regulated by factors that control their ability to bind and hydrolyse guanosine triphosphate (GTP) to guanosine diphosphate (GDP). So far, it has been the general assumption that a GTPase is active or "on" when it is bound to GTP and inactive or "off" in complex with GDP. The GTPases are therefore sometimes referred to as molecular "switches."

The bacterial translational elongation factor EF-Tu is a GTPase, which plays a crucial role during the synthesis of proteins in bacteria, as the factor transports the amino acids that build up a cell's proteins to the cellular protein synthesis factory, the ribosome. Previous structural studies using X-ray crystallography have shown that EF-Tu occurs in two markedly different three-dimensional shapes depending on whether the factor is "on" (i.e. bound to GTP) or "off" (i.e. bound to GDP) (Figure 2). The binding of GTP/GDP have therefore always been thought to be decisive for the factor's structural conformation.

However, a research collaboration between researchers from the Department of Molecular Biology and Genetics at Aarhus University and two American universities reveals that EF-Tu's structure and function, and probably also those of other GTPases, are far more complex than previously assumed. In Søren Thirup's group, X-ray crystallographic analysis of *E. coli* EF-Tu has shown that EF-Tu bound to a variant of GTP, GDPNP, can also occur in the "off" state, which is characterised by a more open structure. In collaboration with American researchers, Charlotte Knudsen's Ph.D. student, Darius Kavaliauskas, conducted further studies using a special form of fluorescence microscopy that makes it possible to observe the spatial structure of individual EF-Tu

molecules in solution.

EF-Tu was labeled with a fluorescence donor and a fluorescence acceptor. When the donor is irradiated with light of a certain wavelength, the light will be absorbed and converted to light with a new wavelength. The acceptor will capture the light and reemit it at a third wavelength, if it is in close proximity to the donor. The transmitted light is measured in a confocal microscope, whereby the distance between donor and acceptor in the EF-Tu molecules can be determined for thousands of molecules in solution, thereby providing information about the dynamic aspects of EF-Tu.

GTPases are far more dynamic than assumed

The study showed that EF-Tu in solution is not in a fixed structure, when the factor is bound to GDP or variations with GDPNP, and thus should be "off" or "on," respectively. Instead, EF-Tu turned out to be extremely dynamic by appearing as a mixture of structures. This tendency was most pronounced when GDPNP was included in the solution, in accordance with the X-ray crystallographic study. Only when binding to the ribosome, EF-Tu assumed the expected active form (Figure 2, left).

The results indicate that in the future, GTPases should be regarded as much more flexible molecules that are not only "on" or "off." GTPases are obvious drug targets: as examples, bacterial infections can in principle be cured by inhibition of EF-Tu, while the GTPase ras p21 is misregulated in approximately 30 percent of all cancers—especially the particularly fatal forms in the lung, colon and pancreas. However, so far it has not been possible to develop a usable drug against these two targets, but the discovery of the high flexibility of the GTPases may help to change this.

More information: Jesper S Johansen et al, E. coli elongation factor

Tu bound to a GTP analogue displays an open conformation equivalent to the GDP-bound form, *Nucleic Acids Research* (2018). [DOI: 10.1093/nar/gky697](https://doi.org/10.1093/nar/gky697)

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