Protein semisynthesis to dissect cellular regulation by post-translational modifications

¶

Beat Fierz

¶

[1]*LCBM, ISIC, Ecole polytechnique fédérale de Lausanne (EPFL), Switzerland*

¶

¶

Post-translational modifications (PTMs) of proteins are critical for the regulation of many cellular processes, including in genome organization via chromatin or in the cytoskeleton, in particular in the regulation of the microtubule network. We have developed methods to dissect chromatin signaling on the single-molecule scale, combining chemical biology approaches and mechanistic biophysics. In particular, we have developed single-molecule fluorescence approaches to directly observe chromatin dynamics as well as to monitor protein interaction dynamics with modified chromatin fibers in real-time.

Recently, we have started to apply similar methods to investigate the regulation of the microtubule network. Chemical, mechanical and dynamic properties of microtubules are important for a host of key cell function, including cell division, intracellular transport, and the establishment of cell polarity. For this functional diversity, control of the microtubule network organization is essential and has to be tightly regulated. Post-translational modifications (PTMs) of tubulin proteins (including poly-glutamylation and detyrosination) play a major role in this regulation, leading to the proposal of a ‘tubulin code’. However, tools to dissect this regulatory system have been lacking. We thus developed a semi-synthesis method, based on sortase- and intein-mediated tandem transamidation, to produce tubulins carrying defined PTMs within their C-terminal tails. Using these designer-tubulins, we show that polyglutamylation of alpha-tubulin stimulates its detyrosination by vasohibin/SVBP. This stimulation is gradual and depends on the length of polyglutamyl chains. Moreover, modulating polyglutamylation levels in cells results in direct and corresponding changes in detyrosination. Together, this directly links the tyrosination-detyrosination cycle to polyglutamylation, connecting the two regulatory systems and controlling tubulin function.