# Post-Translational Tyrosine Phosphorylation Bursts Cytochrome c Dynamics.

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Cytochrome *c* is a key modulator of life-death signaling in mammalian and plant cells[1-3]. Accordingly, its post-translational modifications relate to diverse pathological situations. Indeed, phosphorylation of tyrosine 48 (Tyr48) occurs upon ischemia-reperfusion injury[4]. Hence, the structural and functional features affected by this modification were analyzed through the replacement of Tyr48 with the non-canonical amino-acid *p*-carboxymethyl-phenylalanine (*p*CMF). Notably, analysis of thermal unfolding suggests a destabilization of the weakest folding unit of the protein, which houses the mutation[5].

2D- and 3D-NMR spectra were recorded to assign signals of reduced Y48*p*CMF cytochrome *c* species. NOE integration, distance geometry analysis and restrained molecular dynamics allowed for the modelling of the protein's 3D structure[6], which is similar to that reported for the wild-type species[7]. Nevertheless, the two structures differ at the loop containing the amino-acid substitution and its surroundings. Analysis of relaxation rates and amide heteronuclear NOE values indicate enhanced dynamics around the mutation site. Appropriately, hydrogen exchange experiments indicate an increase of solvent accessibility in this region upon mutation. Strikingly, these changes affect well-known functional sites of cytochrome *c*. Thus, the data offer a hint about how Tyr48 phosphorylation affects diverse physiological processes.

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