**A biophysical approach to study an orphan disease: the case of cblC, a rare inborn disorder of vitamin B12 intracellular metabolism**

MMACHC is a crucial protein in the metabolism of vitamin B12 (cobalamin, Cbl), transforming it into its bioactive forms (AdoCbl and MeCbl), which are cofactors in important cellular reactions. Mutations in the gene coding for MMACHC protein are responsible for the metabolic disorder methylmalonic aciduria and homocystinuria cblC type, which affects children causing neurocognitive and cardiovascular dysfunctions. Although the crystal structure of the wild type protein is available, many molecular features of MMACHC physiopathology remain to be understood and a systematic study on the effect of each specific mutation on the resulting protein is still lacking.

Hence, by using biophysical methods including spectroscopy, microcalorimetry and molecular dynamics we investigated the differences in stability, binding and functionality between MMACHC wild type and the pathological R161Q mutant. Moreover, we evaluated whether non-specific stabilizers (osmolytes) could restore the functionality of the mutant, and performed a virtual screening of chemical libraries to identify pharmacological chaperones to be tested *in vitro.*

Altogether, our findings demonstrated how the combined use of computational and experimental biophysical approaches deepen the knowledge of the molecular mechanisms underlying the function of MMACHC and provide new insights for potential therapeutic interventions.