Investigation on MMACHC-R161Q pathological mutant from cblC disease

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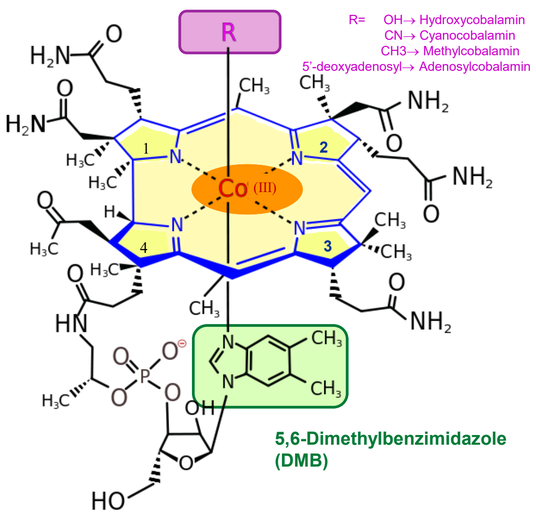
Background

❖ The cblC disease is a rare disorder **of vitamin B12 (cobalamin, Cbl) metabolism** characterized by the abnormal accumulation of methylmalonic acid and homocysteine.

❖ The illness is caused by mutations in the **gene coding for MMACHC** [1], a 282 aa protein that transports and transforms the different forms of Cbl.

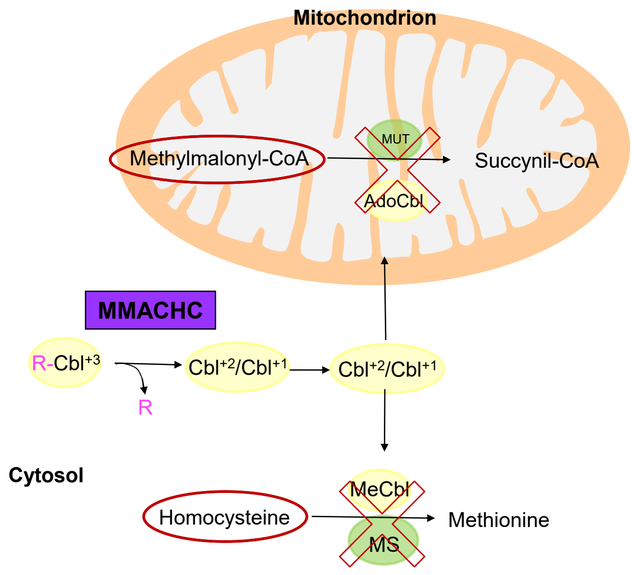
❖ Although the **crystal structure of the wild-type (WT) protein** is available [2,3], many molecular features of MMACHC physiopathology remain to be understood and a systematic study on the effect of each specific mutation on the resulting protein is still lacking.

Cobalamin (Cbl) structure

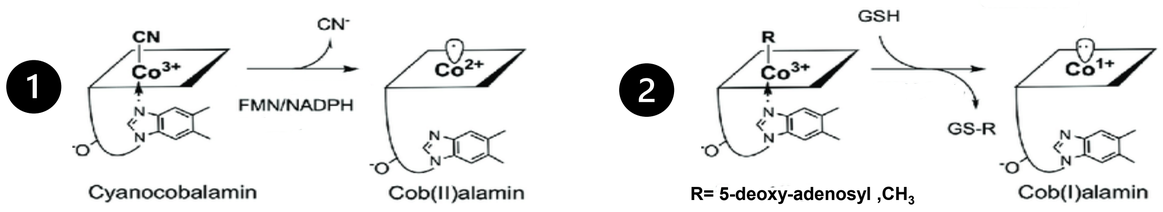


Cobalamin is a complex biomolecule consisting of a central cobalt atom surrounded by a corrin ring with various side chains.

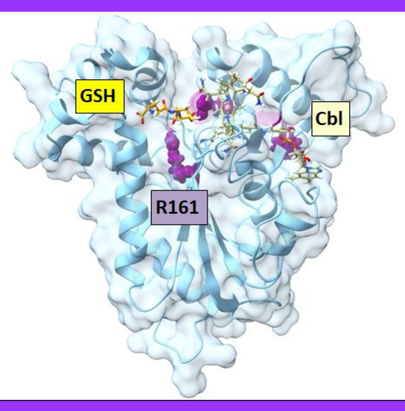
Intracellular Cbl metabolism



MMACHC has the role to transport and process (by reaction 1 or 2) the different forms of Cbl.



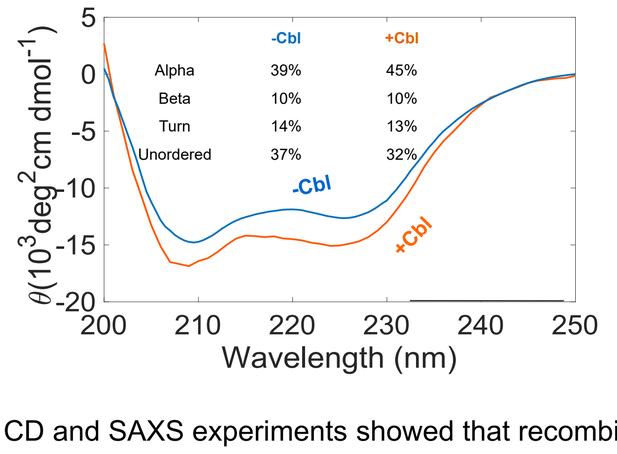
Research goal



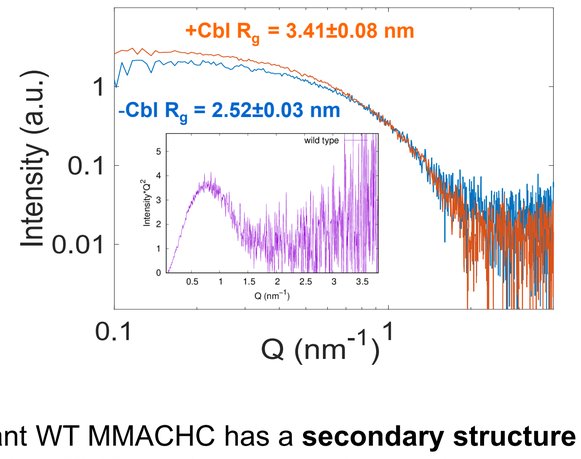
Our aim is to characterize the impact of pathological mutations on MMACHC molecular properties, such as structure, stability, substrates/cofactors binding and catalytic activity, in order to provide a strong basis for further therapeutic development.
Here we focus on MMACHC wild type and **p.R161Q,** a missense mutation that resides in the **GSH** binding pocket, associated with the late-onset disease.

WT protein structure [4]

Circular Dichroism (CD)



Small Angle X-ray Scattering (SAXS)



CD and SAXS experiments showed that recombinant WT MMACHC has a **secondary structure** in good agreement with the crystal structure of the protein [2,3] and it is **compact** in solution. Structural rearrangement occurring upon the **protein-vitamin binding** could be detected.

References

[1] J. P. Lerner-Ellis *et al.*, *Nat Genet* 38, 93-100 (2006).
[2] M. Koutmos, C. et al., *J Biol Chem* 286, 29780-29787 (2011).
[3] D. S. Froese *et al.*, *J Biol Chem* 290, 29167-29177 (2012).
[4] R. Passantino *et al.*, BBA Proteins Proteom 1870(6), 140793 (2022).

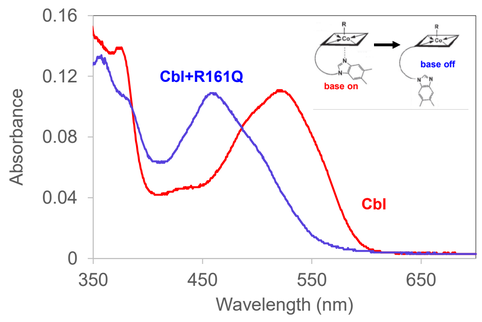
Thanks to

cblC onlus Associazione Italiana Acidemia Metilmalonica con Omocistinuria

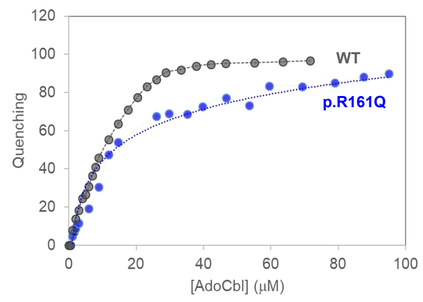
HCU Network America

R161Q mutant

Binding to Cbl

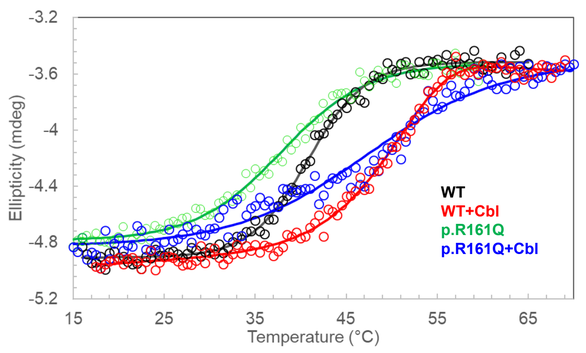


R161Q is **able to bind Cbl**, as shown by the shift 530 nm →460 nm in Cbl absorbance maximum and related to base-on to base-off state transition due to the binding to MMACHC.



However, the fluorescence quenching as a function of AdoCbl concentration revealed **less affinity** to the ligand for the mutant in comparison to WT.

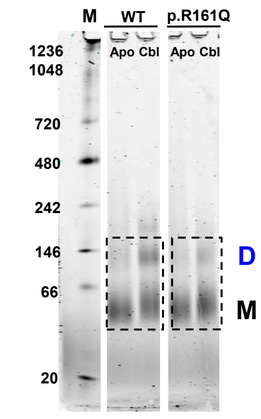
Protein stability



	Apo	+AdoCbl
Wild type	Tm = (41.2±0.1)	Tm = (48.4±0.1)
p.R161Q	Tm = (37.7±0.1)	Tm = (40.8±0.1)

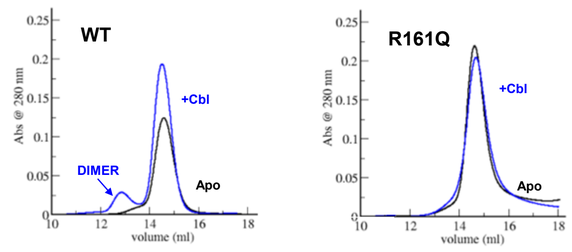
Both WT and p.R161Q in their apo form are **thermolabile** proteins. Moreover, p.R161Q is **less stable** than WT and shows **lower gain in stability** due to the Cbl binding.

Dimer formation



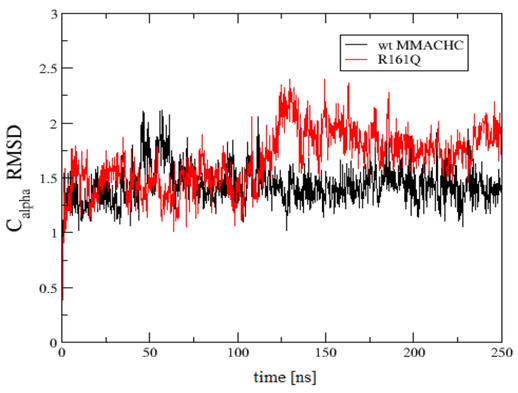
<Mw> (kDa) Light Scattering

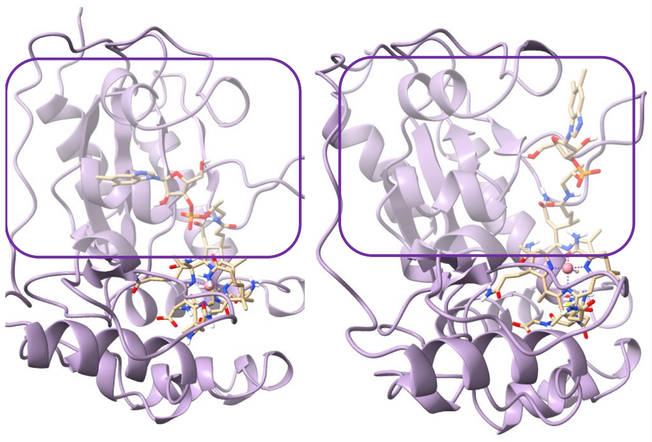
	Apo	+AdoCbl
WT	46.68 ± 0.07	60.82 ± 0.06
p.R161Q	41.75 ± 0.06	41.75 ± 0.14



As shown by FPLC, <MW> values from Light Scattering and native gel electrophoresis, p.R161Q is **less able to form dimers** in the presence of AdoCbl than WT.

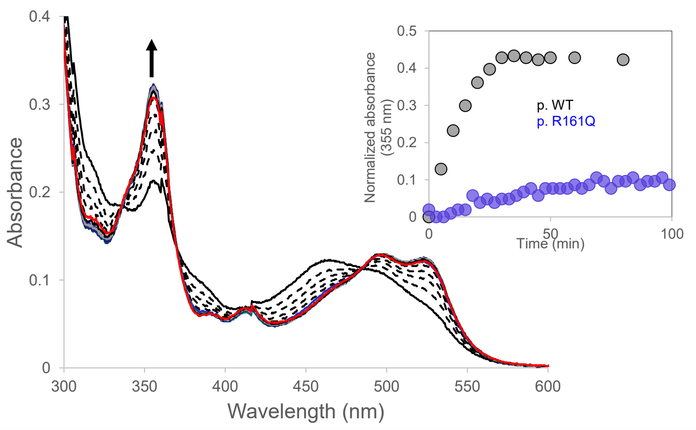
Molecular dynamics





The comparison between the RMSD of the two simulations shows an **abrupt deviation** for the mutant at about 100 ns. This sudden increase, absent in WT protein, is associated to the **extraction of the DMB** tail of the Cbl out of its binding pocket, which is **far** from where the mutation actually occurs.

Functionality assay



Addition of GSH to MMACHC WT with MeCbl resulted in the formation of OH_2Cbl as revealed by the appearance of the characteristic peak at 355 nm in the Cbl absorbance spectrum. No similar behavior could be observed with MeCbl incubated with the mutant p.R161Q, indicating only a **residual functionality**.

Conclusions

❖ The mutation **R161Q affects** not only the region of the mutated residue, but impairs also the **protein global stability**, with consequences on Cbl binding, dimer formation and functionality.

❖ More generally, our results reveal how a **biophysical approach** based on the complementarity of **computational and experimental methods** can offer new insights in the study of the specific effects of the pathological mutations and help prospecting new routes for the cblC treatment.