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BLUE CRAB (*CALLINECTES SAPIDUS*) AS A SOURCE OF BIOACTIVE COMPOUNDS: HOW TO TURN A PROBLEM INTO A RESOURCE

IL GRANCHIO BLU (*CALLINECTES SAPIDUS*) COME FONTE DI COMPOSTI BIOATTIVI: COME CONVERTIRE UN PROBLEMA IN RISORSA

Abstract - Invasive species threaten marine biodiversity, together with overfishing and climate change. A striking case is the blue crab *Callinectes sapidus*, whose spread in the Mediterranean Sea disrupts ecosystems and damages coastal economies. Turning such invaders into valuable resources may help controlling their populations: the greater their market value, the stronger the interest for their exploitation. This study explores blue crab valorization by focusing on bioactive molecules, particularly carotenoproteins, within a biorefinery framework aimed at near-zero waste production. β -crustacyanin, the main shell carotenoprotein, is an eight-heterodimer complex binding two astaxanthin molecules. To assess its potential, we performed molecular docking and dynamics simulations showing that astaxanthin strongly inhibits SARS-CoV-2 main protease (Mpro) forming a stable complex. These promising results suggest blue crab carotenoproteins as potential candidates for industrial and therapeutic applications, including anti-COVID-19 strategies.

Keywords: *Callinectes sapidus*, β -crustacyanin, SARS-CoV-2 protease.

Introduction - The blue crab *Callinectes sapidus* Rathbun, 1896 native in the Atlantic Sea and in the Gulf of Mexico, is now an invasive species in the Mediterranean, threatening ecosystems and biodiversity (Clavero *et al.*, 2022). While appreciated for its meat, its processing waste, especially shells, remain underexploited. Rich in chitin, minerals, and bioactive pigments, shells offer opportunities for valorization, which could also help curb crab populations through commercial use (Tamburini, 2024).

The main pigment of *C. sapidus* is the crustacyanin, a complex between a protein and a carotenoid (astaxanthin). Astaxanthin's uses span from food and feed dyeing to nutraceuticals applications due to its strong antioxidant activity (Šimat *et al.*, 2022). In blue crab shells, crustacyanin occurs as α -crustacyanin, a complex of eight β -crustacyanin heterodimers, each binding two astaxanthin molecules, for a total of sixteen. It stores large amounts of astaxanthin and causes the shell's bathochromic blue shift upon heating (Cianci *et al.*, 2002). β -crustacyanin, a member of the lipocalin family, is specialized in storing and transporting hydrophobic molecules (Fig. 1).

In this work, we adopt a computational approach (Molecular Docking and Molecular Dynamics) to verify the activity of the astaxanthin molecule as potential inhibitor of the Main Protease (Mpro) of the SARS-CoV-2, the virus responsible for the COVID19 pandemics.

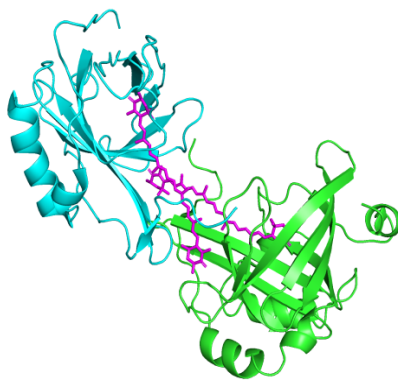


Fig. 1 – Molecular structure of β -crustacyanin from *Homarus gammarus* (Linnaeus, 1758) (source Protein Data Bank, PDB code 1GKA). The two chains of the heterodimer are shown in cyan and green, the two molecules of astaxanthin are shown in magenta sticks.

Struttura molecolare della β -crostacianina di Homarus gammarus (Linnaeus, 1758) (fonte: Protein Data Bank, codice PDB 1GKA). Le due catene dell'eterodimero sono mostrate in ciano e verde, le due molecole di astaxantina sono mostrate in magenta.

Figure 2 shows the molecular structure of SARS-CoV-2 Mpro (Zhang *et al.*, 2020), a homodimer where each chain has three domains. The catalytic residues lie in two different domains, while the third mediates dimerization, essential for protease activity, as monomers are inactive.

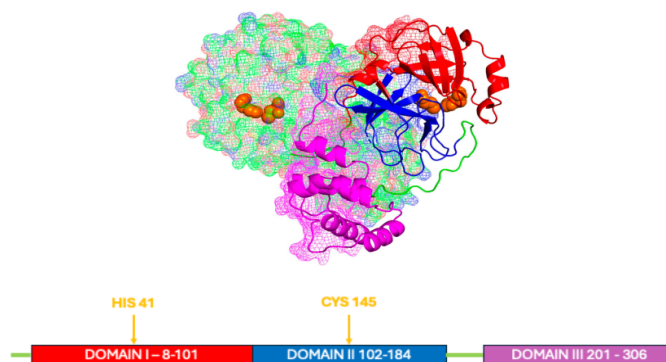


Fig. 2 – Molecular structure (homodimer) of SARS-CoV-2 main protease (Mpro): in red, blue and magenta are shown the domains included in each chain. In orange are indicated the two residues of the catalytic site. The magenta domain is responsible for the Mpro dimerization (source Protein Data Bank, PDB code 6Y2E).

Struttura molecolare (omodimero) della proteasi principale di SARS-CoV-2 (Mpro): in rosso, blu e magenta sono mostrati i domini inclusi in ciascuna catena. In arancione sono indicati i due residui del sito catalitico. Il dominio magenta è responsabile della dimerizzazione di Mpro (fonte Protein Data Bank, codice PDB 6Y2E).

Materials and methods – In our computational analysis the molecular target was the SARS-CoV-2 main protease (PDB code 6Y2E) and the ligand in the Molecular Docking was the astaxanthin. The computational methods applied to compute and analyze the Mpro-astaxanthin complex were:

- Molecular Docking by AUTODOCK Vina, rigid docking applied by means of the DockingPie plugin of Pymol to derive the first conformation of the Mpro-astaxanthin complex (Rosignoli & Paiardini, 2022);

- **Molecular Dynamics:** GROMACS at T= 300 K in physiological solution (NaCl 0.15 M) for the relaxation of the Mpro-astaxanthin complex using as starting conformation the output of the Molecular Docking (Abraham *et al.*, 2015).

The general analysis of the protein molecular structure and of protein-protein interface was performed using the PISA web server (<https://www.ebi.ac.uk/pdbe/pisa/>) deriving different thermodynamic parameters detailed in Hadi-Alijanvand *et al.* (2022). In detail, given a complex made of n subunits A_1, A_2, A_n , the free energy of dissociation ΔG_{diss} is defined as:

$$\Delta G_{diss} = -\Delta G_{int} - T\Delta S_{diss}$$

where ΔG_{int} is the binding energy of the subunits A_i , ΔS_{diss} is the entropy variation upon dissociation. Unstable protein complexes correspond to $\Delta G_{diss} < 0$ and $T\Delta S_{diss}$ describes the loss of entropy ($T\Delta S_{diss} < 0$) of solvent molecules upon complex formation. Positive values of ΔH_{diss} show favourable solvation, so the decrease of ΔH_{diss} upon binding indicates an increase of the molecule hydrophobicity with respect to the unbound form. The binding energy of the subunits ΔG_{int} includes contributions for the solvation of the complex, the contact-dependent (short-range) and the electrostatic (long-range) interactions between chains.

Results – The binding with the astaxanthin destabilizes the Mpro structure, so it is possible to argue its activity as inhibitor of the Mpro activity. It is noteworthy the astaxanthin molecule undergoes a bending to fit into a cavity between the Domain II and III, possibly hindering the dimerization and the enzyme function.

Fig. 3 reports the Molecular Docking and Molecular Dynamics outputs, showing the estimated Mpro-Astaxanthin complex. Tab. 1 reports the stability properties of the two forms of the Mpro (apo and holo).

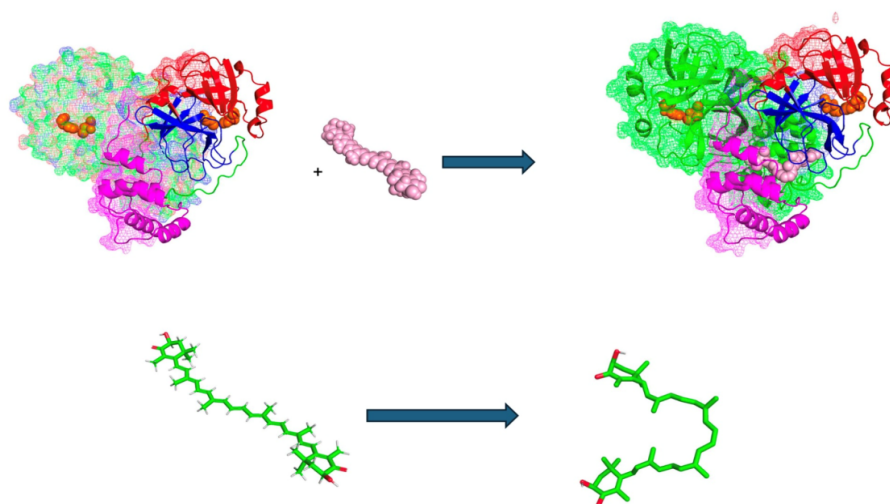


Fig. 3 – The molecular structure of the MPro-Astaxanthin complex.
La struttura molecolare del complesso MPro-Astaxantina.

Tab. 1 – Stability properties of the apo and holo forms of the MPro.
Proprietà di stabilità delle forme apo e olo del MPro.

MPro form	ΔG_{diss} kcal/mole	$T\Delta S_{\text{diss}}$ kcal/mole	ΔH_{diss} kcal/mole
Apo	19.8	13.9	33.7
Holo (+ astaxanthin)	1.6	14.1	15.7

The two forms of MPro are both stable, with the apo (unbound) form being more stable than the holo (bound to astaxanthin). The bound form is less stable since the hydrophobic ligand (astaxanthin) reduces the enthalpic contribution to stabilization (ΔH_{diss}).

Conclusions - This study highlights the dual potential of *Callinectes sapidus*: while invasive in the Mediterranean, it represents a valuable resource within a biorefinery approach. Computational analyses show that astaxanthin, carried by shell carotenoproteins, can bind and destabilize SARS-CoV-2 main protease, suggesting its inhibitory activity. These results support blue crab valorization both for ecosystem management helping to solve the threat related to crustaceans' waste and as a source of bioactive compounds with promising industrial and therapeutic applications. While uses of crustaceans' by-products have already been documented (Tamburini, 2024), this is the first report on the use of astaxanthin as an inhibitor of SARS-CoV-2, turning the problem of blue crab invasion into a potential resource for the field of blue biotechnologies.

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