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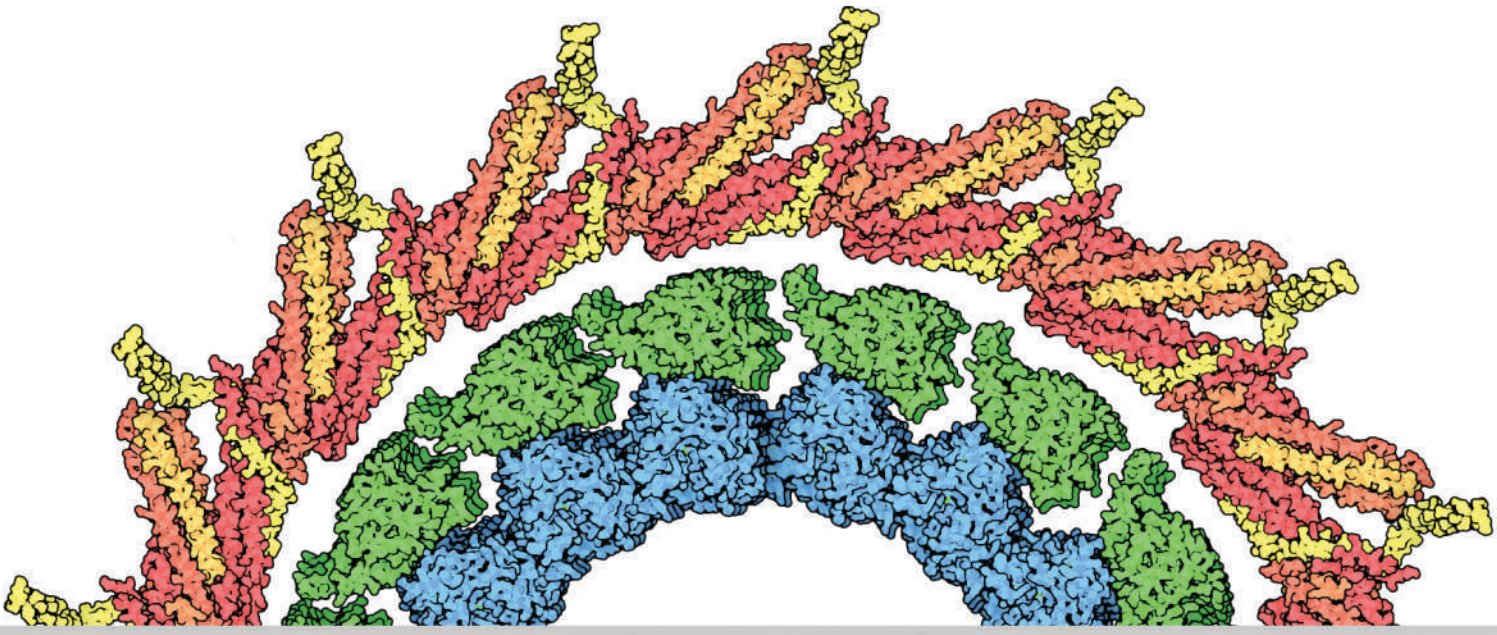

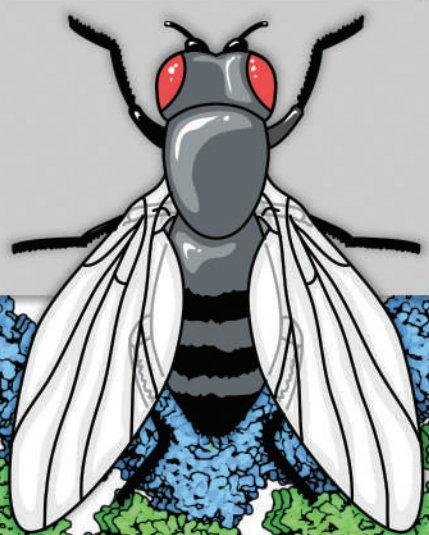

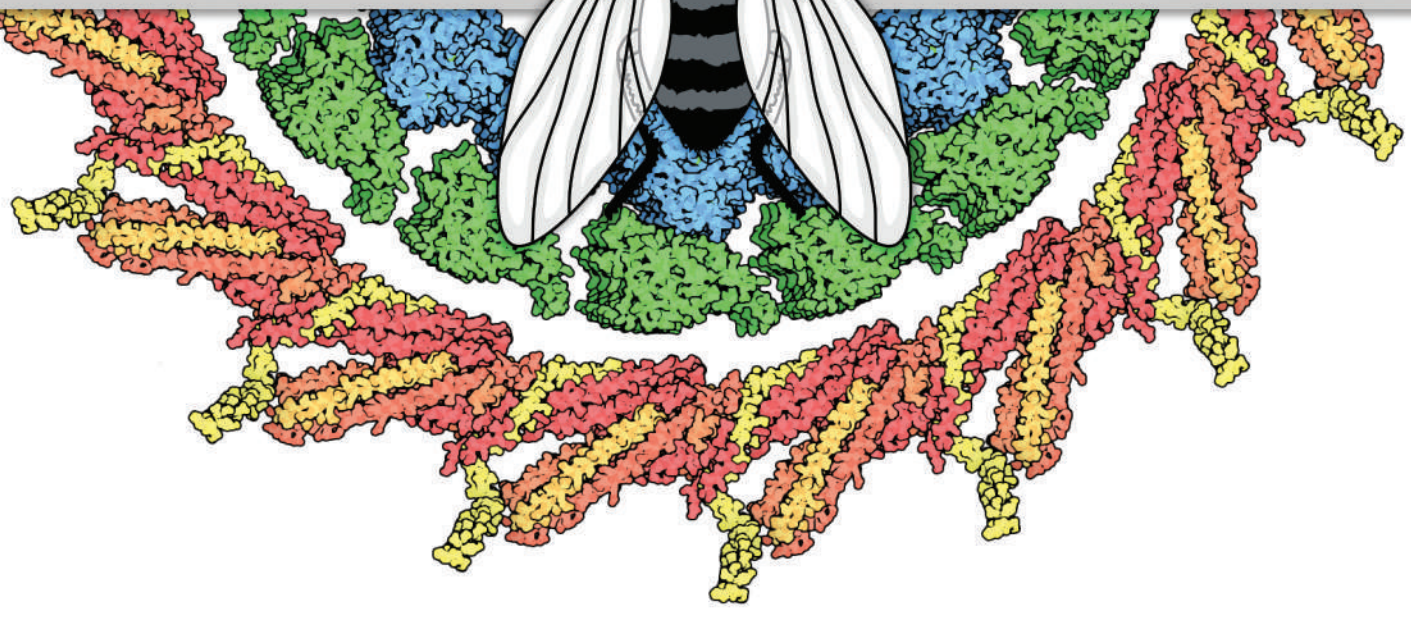

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Design and Testing of a Colorimetric Immunoassay for the Measurement of Phenylalanine Blood Levels in Patients with Phenylketonuria

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Phenylketonuria (PKU) is an inborn metabolic disease that affects 1 out of every 10 000 people in Europe and leads to brain retardation and other neurophysiological problems if it's not adequately treated. PKU is caused by mutations in the gene that codes for phenylalanine hydroxylase (PAH), an enzyme that catalyzes the transformation of the amino acid phenylalanine into tyrosine in hepatocytes. These mutations trigger the misfolding of the tetrameric native state of PAH and, consequently, a decrease in enzymatic activity, leading to high blood phenylalanine levels that are toxic to brain cells. Routine controls of phenylalanine blood levels in patients are carried out by mass spectrometry (MS/MS) in hospitals and specialized laboratories, so it delays the obtaining of results. In order to improve this fact, we aim to design a kit that allows for the measurement of the amino acid concentration at home in a rapid, reliable and economical way. For this purpose, we took advantage of a previously identified pharmacological chaperone (compound PCIV) of PAH and chemically derivatized it in order to develop a colorimetric ELISA-sandwich immunoassay based on the competition between phenylalanine and PCIV for the interaction with PAH in the active site of the protein. In this system, the spectroscopically determined colorimetric signal is sensitive to phenylalanine concentration in aqueous samples. However, the range of signal variation is very short and, combined with the presence of non-specific interactions, this make our assay insufficient to get an accurate quantification of the amino acid concentration. This drawback could be solved through the use of a compound with stronger binding affinity for PAH than PCIV, giving rise to a more specific interaction and, finally, leading to a more reliable test that can help PKU patients to deal with the disease.

Keywords

Phenylketonuria | ELISA | Colorimetric Immunoassay | PAH