**Study of the anthocyanin-protein interaction and its role in anthocyanin blue color stabilization.**

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Anthocyanins are phenolic compounds responsible for the red, violet, and blue colors found in flowers, fruits, and legumes1. However, the color presented by anthocyanins is only stable at acidic (pH<2) conditions, which limits their use in different applications. Nature has developed strategies to stabilize the blue form of anthocyanins in neutral or slightly acidic pH conditions. Specifically, the blue color observed in certain flowers arises from the complexation of the quinoidal base of anthocyanins with metals such as Mg²⁺ and Fe³⁺,1, 2 as well as intramolecular co-pigmentation, which involves a sandwich-type stacking between aromatic acyl residues and the quinoidal base chromophore of polyacylated anthocyanins3. Although blue color is commonly found in nature, it remains challenging to replicate in food products. Recently, Denish *et al.*, 2021 used a multidisciplinary approach, including computational simulations and synthetic biology, to develop a natural alternative to the synthetic dye FD&C Blue No. 1 based on the complexation of an anthocyanin from red cabbage with Al3+.4 This discovery could lead to safer, more sustainable food colorants, addressing a long-standing challenge in the industry​.

More recently, Wang *et al.*, showed that the blue color of the six most common non-acylated anthocyanins can be stabilized using bovine and human serum albumins and lysozyme at pH 7.5

In this context, different plant-based proteins (e.g., pea and rice) will be used to study their interaction with different anthocyanins, aiming to stabilize their blue color. For this purpose, techniques such as ultraviolet-visible spectroscopy (UV-Vis), fluorescence, isothermal titration calorimetry (ITC), nuclear magnetic resonance (NMR), among others, will be employed.

**References**

1. Cruz, L.; Basílio, N.; Mateus, N.; de Freitas, V.; Pina, F., Natural and Synthetic Flavylium-Based Dyes: The Chemistry Behind the Color. *Chemical Reviews* **2022,** *122* (1), 1416-1481.
2. Mendoza, J.; Basílio, N.; Pina, F.; Kondo, T.; Yoshida, K., Rationalizing the Color in Heavenly Blue Anthocyanin: A Complete Kinetic and Thermodynamic Study. The Journal of Physical Chemistry B 2018, 122 (19), 4982-4992.
3. Terahara, N.; Oda, M.; Matsui, T.; Osajima, Y.; Saito, N.; Toki, K.; Honda, T., Five New Anthocyanins, Ternatins A3, B4, B3, B2, and D2, from Clitoria ternatea Flowers. Journal of Natural Products 1996, 59 (2), 139-144.
4. Denish Pamela, R.; Fenger, J.-A.; Powers, R.; Sigurdson Gregory, T.; Grisanti, L.; Guggenheim Kathryn, G.; Laporte, S.; Li, J.; Kondo, T.; Magistrato, A.; Moloney Mícheál, P.; Riley, M.; Rusishvili, M.; Ahmadiani, N.; Baroni, S.; Dangles, O.; Giusti, M.; Collins Thomas, M.; Didzbalis, J.; Yoshida, K.; Siegel Justin, B.; Robbins Rebecca, J., Discovery of a natural cyan blue: A unique food-sourced anthocyanin could replace synthetic brilliant blue. Science Advances 7 (15), eabe7871.
5. Wang, W., Yang, P., Gao, F., Wang, Y., Xu, Z., & Liao, X. (2024, Feb 24). Metal-free production of natural blue colorants through anthocyanin-protein interactions. *J Adv Res*. <https://doi.org/10.1016/j.jare.2024.02.018>

1. Denish Pamela, R.; Fenger, J.-A.; Powers, R.; Sigurdson Gregory, T.; Grisanti, L.; Guggenheim Kathryn, G.; Laporte, S.; Li, J.; Kondo, T.; Magistrato, A.; Moloney Mícheál, P.; Riley, M.; Rusishvili, M.; Ahmadiani, N.; Baroni, S.; Dangles, O.; Giusti, M.; Collins Thomas, M.; Didzbalis, J.; Yoshida, K.; Siegel Justin, B.; Robbins Rebecca, J., Discovery of a natural cyan blue: A unique food-sourced anthocyanin could replace synthetic brilliant blue. *Science Advances 7* (15), eabe7871.