Japan Academy Prize to:

Kumi Yoshida
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Visiting Professor, Faculty of Engineering,
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for "Studies on Mechanisms of Blue Flower Coloration by Anthocyanins"

Outline of the work:

The captivating colors of flowers, which range from blue and purple to red, have an important aesthetic and cultural significance for humans throughout the world. Anthocyanins are pigment molecules responsible for much of this coloration. Chemically, anthocyanins are flavonoid compounds characterized by specific sugar chains and organic acids that are attached to chromophore structures. Scientific investigation of flower color chemistry originated in the late 19th century. However, by the early 20th century, a notable scientific debate emerged between German and Japanese chemists over the "pH theory" versus the "metal complex theory" as an explanation for anthocyanin-based blue flower coloration. Moreover, this controversy remained unresolved for a long time. Given this context, Dr. Kumi Yoshida developed a keen interest in understanding the mechanistic basis of blue flower coloration, and approached this issue from the perspective of natural product chemistry. She pursued research methodologies that integrated organic chemistry with plant physiology, aiming to elucidate both the structural diversity of blue pigments and the variety of mechanisms responsible for the multiple coloration forms produced by anthocyanins. In general, Dr. Yoshida's contributions can be categorized into three primary areas. Key results in each of these are described below.

I) Research on blue flower coloration caused by metal complex anthocyanins in dayflowers and other plants

First, Dr. Yoshida investigated a blue pigment commelinin, that is present in the blue dayflower, Tsuyukusa. To do so, she purified anthocyanin and flavone components of commelinin, then reconstituted a supramolecular metal complex pigment known as metalloanthocyanin (commelinin) by combining these components with specific metal ions. Using X-ray crystallographic analysis of a single commelinin crystal, Dr. Yoshida was the first to elucidate the supramolecular structure of commelinin, marking a major breakthrough. Her findings demonstrated that anthocyanins complex with metal ions to produce a blue color, providing robust evidence for the metal complex theory. Dr. Yoshida further showed that the blue coloration observed in cornflower, salvia, and nemophila species is also generated by supramolecular metal complexes that are analogous to commelinin. Moreover, these metalloanthocyanins reliably self-assemble in mixtures of anthocyanins, flavonoids, and metal ions such as magnesium.

II) Research on blue flower color and color changes of polyacrylate anthocyanins, including in morning glory

In blue flowers of morning glory (e.g., *Ipomoea* spp.), buds are initially red but turn blue as the flower blooms. During this transition, the structure of petal anthocyanin remains unchanged, suggesting that changes in pH drive the color shift. In petals, surface cells contain color, while inner cells remain colorless. Accordingly, a pressed extract of the blue petals is known to turn purple. To study this unique system, Dr. Yoshida applied an innovative intracellular microelectrode technique to measure vacuolar pH, specifically in surface cells. She successfully measured the vacuolar pH in red buds as approximately 6.6, although it increased to 7.7 when petals turned blue as they opened. Overall, this pH shift strongly supports the pH theory. To investigate the mechanism responsible for increasing pH, Dr. Yoshida demonstrated that within the 24 h window immediately prior to flowering, potassium ions enter plant vacuoles, thereby drawing in water and resulting in a higher pH. This discovery has profoundly impacted plant physiology by uncovering a universal mechanism for vacuolar pH adjustment that is tied to cellular growth.

III) Research on blue pigments and color variation in hydrangea

Hydrangeas native to Japan were originally known to display blue sepals; however, their color is highly sensitive to soil conditions. For example, when grown in acidic soils, hydrangea sepals can turn blue, whereas in neutral or basic soils, they appear red. Research in the early 20th century showed that identical organic compounds are present in both blue and red sepals, with aluminum ions contributing to the blue color. Nevertheless, the structure of the blue pigment and the mechanism of the color change remain unknown. Using advanced chemical investigations, Dr. Yoshida was the first to identify the blue pigment as a 1:1:1 complex of anthocyanin, neochlorogenic acid, and aluminum ions. Moreover, she also observed that in purple hydrangea sepals, colored cells form a mosaic of blue, purple, and red colors. To elucidate this phenomenon, Dr. Yoshida prepared protoplasts from each color type and collected samples for microscopic analysis. Using ultra-microanalytical techniques, she revealed that slight changes in the ratio of aluminum ions to anthocyanin, variation in the composition of copigments, and vacuolar pH can all significantly influence the formation of the hydrangea blue complex, thereby driving color variation.

In summary, Dr. Yoshida has pioneered innovative research methods rooted in her original analyses of flower coloration, and has elucidated fundamental mechanisms that answer long-standing scientific questions. To date, her work has provided definitive answers to questions that have intrigued researchers for more a century. Furthermore, Dr. Yoshida's scholarly contributions are highly esteemed across multiple scientific disciplines, since her work offers significant insight into the complex chemistry underlying natural pigments. Dr. Yoshida has also been at the forefront of her field regarding international collaboration, as she has organized two symposia in Japan focused on floral coloration research. In recognition of her impactful work, Dr. Yoshida has been honored with a JSBBA Award from the Japan Society for Bioscience, Biotechnology, and Agrochemistry, as well as a Scientific Prize from Groupe Polyphenols, the preeminent global organization for polyphenol research.

List of Main Publications

I) Research on blue flower coloration caused by metal complex anthocyanins in dayflowers and other plants

[Research on the structure of blue petal pigments in dayflowers]

1. Goto, T., Yoshida, K., Yoshikane, M. and Kondo, T. Chiral stacking of a natural flavone, flavocommelin, in aqueous solutions. *Tetrahedron Lett.*, **31**, 713–716 (1990).
2. Kondo, T., Yoshida, K., Yoshikane, M. and Goto, T. Mechanism for color development in purple flower of *Commelina communis*. *Agric. Biol. Chem.*, **55**, 2919–2921 (1991).
3. Kondo, T., Yoshida, K., Nakagawa, A., Kawai, T., Tamura, H. and Goto, T. Structural basis of blue-colour development in flower petals: structure determination of commelinin from *Commelina communis*. *Nature*, **358**, 515–518 (1992).

[Blue coloration by supramolecular metal complex pigments: metalloanthocyanins (commelinin)]

4. Kondo, T., Ueda, M., Yoshida, K., Titani, K., Isobe, M. and Goto, T. Direct observation of a small-molecule associated supramolecular pigment, commelinin, by electrospray ionization mass spectroscopy. *J. Amer. Chem. Soc.*, **116**, 7457–7458 (1994).
5. Kondo, T., Oyama, K-i. and Yoshida, K. Chiral molecular recognition on formation of a metalloanthocyanin: A supramolecular metal complex pigment from blue flower of *Salvia patens*. *Angew. Chem. Int. Ed.* **40**, 894–897 (2001).
6. Mori, M., Kondo T. and Yoshida, K. Cyanosalvianin, a supramolecular blue metalloanthocyanin, from petals of *Salvia uliginosa*. *Phytochemistry*, **69**, 3151–3158 (2008).
7. Yoshida, K., Tojo, K., Mori, M., Yamashita, K., Kitahara, S., Noda, M. and Uchiyama, S. Chemical mechanism of petal color development of *Nemophila menziesii* by a metalloanthocyanin, nemophilin. *Tetrahedron*, **71**, 9123–9130 (2015).
8. Yoshida, K., Teppabut, Y., Sugita, C. and Oyama, K-i. Blue flower coloration of *Salvia macrophylla* by the metalloanthocyanin, protodelphin. *Biosci. Biotech. Biochem.*, **86**, 1349–1352 (2022).

[Expression of blue flower coloration involving metal complexes]

9. Yoshida, K., Kitahara, S., Ito, D. and Kondo, T. Ferric ions involved in the flower color development of the Himalayan blue poppy, *Meconopsis grandis*. *Phytochemistry*, **67**,

992–998 (2006).
10. Shoji, K., Miki, N., Nakajima, N., Momonoi, K., Kato, C. and Yoshida, K. Perianth bottom-specific blue color development in tulip cv. Murasakizuisho requires ferric ions. *Plant Cell Physiol.,* **48**, 243–251 (2007).
11. Momonoi, K., Yoshida, K., Mano, S., Takahashi, H., Nakamori, C., Shoji, K., Nitta, A. and Nishimura, M. A vacuolar iron transporter in tulip, TgVit1, is responsible for blue coloration in petal cells through iron accumulation. *Plant J.*, **59**, 437–447 (2009).
12. Yoshida, K. and Negishi, T. The identification of a vacuolar iron transporter involved in the blue coloration of cornflower petals. *Phytochemistry*, **94**, 60–67 (2013).
13. Yoshida, K., Oniduka, T., Oyama, K-i. and Kondo, T. Blue flower coloration of *Corydalis ambigua* requires ferric ion and kaempferol glycoside. *Biosci. Biotech. Biochem*., **85**, 61–68 (2020).

II) Research on blue flower color and color changes of polyacrylate anthocyanins, including in morning glory

[Measurement of pH in morning glory petal cells and mechanism of flower color change]
1. Yoshida, K., Kondo, T., Okazaki, Y. and Katou, K. Cause of blue petal colour. *Nature*, **373**, 291 (1995).
2. Yoshida, K., Osanai, M. and Kondo, T. Mechanism of dusky reddish-brown "kaki" color development of Japanese morning glory, *Ipomoea nil* cv. Danjuro. *Phytochemistry*, **63**, 721–726 (2003).
3. Yoshida, K., Kawachi, M., Mori, M., Maeshima, M., Kondo, M., Nishimura M. and Kondo, T. The involvement of tonoplast proton pumps and $Na^{+}(K^{+})/H^{+}$ exchangers in the change of petal color during flower opening of morning glory, *Ipomoea tricolor* cv. Heavenly Blue. *Plant Cell Physiol.,* **46**, 407–415 (2005).
4. Yoshida, K., Miki, N., Momonoi, K., Kawachi, M., Katou, K., Okazaki, Y., Uozumi, N., Maeshima M. and Kondo, T. Synchrony between flower opening and petal-color change from red to blue in morning glory, *Ipomoea tricolor* cv. Heavenly Blue. *Proc. Jpn. Acad. Ser. B*, **85**, 187–197 (2009).

[Stabilization of blue coloration by intramolecular stackings of polyacylated anthocyanins]
5. Yoshida, K., Kondo, T. and Goto T. Intramolecular stacking conformation of gentiodelphin, a diacylated anthocyanin from *Gentiana makinoi. Tetrahedron*, **48**, 4313–4326 (1992).
6. Yoshida, K., Toyama, Y., Kameda, K. and Kondo, T. Contribution of each caffeoyl residue of the pigment molecule of gentiodelphin to blue color development. *Phytochemistry*, **54**, 85–92 (2000).
7. Yoshida, K., Okuno, R., Kameda, K., Mori, M. and Kondo, T. Influence of *E*, *Z*-isomerization and stability of acylated anthocyanins under the UV irradiation. *Biochem. Eng. J.*, **14**, 163–169 (2003).
8. Yoshida, K., Mori, M., Kawachi, M., Okuno, R., Kameda, K. and Kondo, T. A UV-B

resistant polyacylated anthocyanin, HBA, from blue petals of morning glory. *Tetrahedron Lett.*, **44**, 7875–7880 (2003).
9. Mori, M., Ito, D., Miki, N., Kondo, T. and Yoshida, K. Structure of tecophilin, a tri-caffeoylanthocyanin from the blue petals of *Tecophilaea cyanocrocus*, and the mechanism of blue color development. *Tetrahedron*, **70**, 8657–8664 (2014).
10. Mendoza, J., Basílio, N., Pina, F., Kondo, T. and Yoshida, K. Rationalizing the color in heavenly blue anthocyanin: A complete kinetic and thermodynamic study. *J. Phys. Chem. B*, **122**, 4982–4992 (2018).

III) Research on blue pigments and color variation in hydrangea

[Elucidation of the structure of blue pigments in hydrangea]

1. Kondo, T., Toyama-Kato Y. and Yoshida, K. Essential structure of co-pigment for blue sepal-color development of hydrangea. *Tetrahedron Lett.*, **46**, 6645–6649 (2005).
2. Toyama-Kato, Y., Kondo, T. and Yoshida, K. Synthesis of designed acylquinic acid derivatives involved in blue color development of hydrangea and their co-pigmentation effect. *Heterocylces*, **72**, 239–254 (2007).
3. Oyama, K-i., Watanabe, N., Yamada, T., Suzuki, M., Sekiguchi, Y., Kondo, T. and Yoshida, K. Efficient and versatile synthesis of 5-*O*-acylquinic acids with a direct esterification using a *p*-methoxybenzyl quinate as a key intermediate. *Tetrahedron*, **71**, 3120–3130 (2015).
4. Oyama, K-i., Yamada, T., Ito, D., Kondo, T. and Yoshida, K. Metal-complex pigment involved in the blue sepal color development of hydrangea. *J. Agric. Food. Chem.,* **63**, 7630–7635 (2015).
5. Ito, T., Oyama, K-i. and Yoshida, K. Direct observation of hydrangea blue-complex composed of 3-*O*-glucosyldelphinidin, Al^{3+} and 5-*O*-acylquinic acid by ESI-Mass Spectrometry. *Molecules*, **23**, 1424 (2018).
6. Ito, T., Aoki, D., Fukushima, K. and Yoshida, K. Direct mapping of hydrangea blue-complex in sepal tissues of *Hydrangea macrophylla*. *Sci. Rep.*, **9**, 5450 (2019).

[Elucidation of the mechanism of color variation in hydrangea]

7. Yoshida, K., Toyama-Kato, Y., Kameda, K. and Kondo, T. Sepal color variation of *Hydrangea macrophyll*a and vacuolar pH measured with a proton-selective microelectrode. *Plant Cell Physiol.*, **44**, 262–268 (2003).
8. Yoshida, K., Ito, D., Shinkai, Y. and Kondo, T. Change of color and components in sepals of chameleon hydrangea during maturation and senescence. *Phytochemistry*, **69**, 3159–3165 (2008).
9. Ito, D., Shinkai, Y., Kato, Y., Kondo, T. and Yoshida, K. Chemical studies on different color development in blue- and red-colored sepal cells of *Hydrangea macrophylla*. *Biosci. Biotech. Biochem.*, **73**, 1054–1059 (2009).
10. Yoshida, K., Ito, D., Miki, N. and Kondo, T. Single-cell analysis clarifies mosaic color development in purple hydrangea sepal. *New Phytologist*, **229**, 3549–3557 (2021).

[Research on acidic soil tolerance in hydrangea]

11. Negishi, T., Oshima, K., Hattori, M., Kanai, M., Mano, Shoji, Nishimura, M. and Yoshida, K. Tonoplast- and plasma membrane-localized aquaporin-family transporters in blue hydrangea sepals of aluminum hyperaccumulating plant. *PLOS One*, **7**, e73189 (2012).
12. Negishi, T., Oshima, K., Hattori, M. and Yoshida, K. Plasma membrane-localized Al-transporter from blue hydrangea sepals is a member of the anion permease family. *Genes Cells*, **18**, 341–352 (2013).