Reflections from a developing butterfly Gyroid

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The occurrence of single Gyroid nanostructures in butterflies is remarkable; not least because of the large unit cell sizes (~300 nm) that allow the structures to behave as three-dimensional biophotonic crystals. The single Gyroid structure has been found in several species of butterflies, generating a striking green coloration. While Gyroid structures have been well documented in other naturally occurring systems, the sizes of the structures found in butterflies are much larger, leading to the question of their formation. In butterflies, single Gyroid nanostructures are found within the lumen of their wing scales. Each scale develops from a single cell whose plasma membrane acts as a template to the nascent chitin, forming elaborate nanostructures. Upon maturation, the scale cell dies and recedes leaving only the chitinous nanostructures. In the butterfly *Callophrys gryenus*, the complex Gyroid network is thought to be templated by an additional intracellular membrane, namely the smooth endoplasmic reticulum (SER). This membrane, along with the plasma membrane are thought to fold into a double Gyroid geometry comprising three distinct channels: the extracellular space, the intracellular space, and the intra-SER space. The prevailing hypothesis is that chitin is deposited into this extracellular space, thereby generating a chitinous single Gyroid structure, and that the remaining channels are filled with cytoplasm and extracellular fluid that are gradually replaced by air as the scale cell recedes [1,2]. Currently, the only evidence to support this hypothesis comes from Ghiradella's seminal TEM study of fixed pupal wing tissue from different developmental stages [3]. The lack of evidence is largely due to the difficulties associated with imaging structures of this size. Their nanometer scale limits imaging techniques to electron microscopy which restricts the sample to one that can be imaged under vacuum (i.e. not a living organism). What is needed to understand the details of the formation mechanism is a technique that allows *in vivo* imaging of a developing butterfly pupa.

Here, we take advantage of the fact that these structures are photonic and measure the reflections that are generated by developing biophotonic Gyroid nanostructures in *Parides sesostris* to infer specific details about their development. We developed and used a hyperspectral microscopy method [4] and electron microscopy to show that the Gyroid nanostructure begins to generate red-shifted reflections peaking ~610 nm at ~75% development. Over the course of ~48 hours these reflections grow and almost triple in amplitude. These red-shifted reflections are commensurate with optical models of a chitinous single Gyroid nanostructure that is embedded within a cytosolic medium and provide further support for the double Gyroid templating hypothesis.

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