A novel method for visualizing the electrostatic complementarity of protein-protein interaction based on fragment molecular orbital method

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Abstract

Here, the development of a method for visualizing the electrostatic complementarity of protein-protein interactions (PPIs) using fully quantum mechanical electron density (EDN) and electrostatic potential (ESP) is described. For this method, the partial EDN (pEDN) and partial ESP (pESP) of each protein were newly defined based on equations used for the fragment molecular orbital method. To demonstrate the efficacy of the method, calculations were performed for the complex of programmed cell death-1 (PD-1) and its ligand (PD-L1). The results showed that the interface between PD-1 and PD-L1 was appropriately determined by the pEDN, and that the electrostatic complementarity of the PPI was clearly represented by visualizing the pESP. Further analysis of the pESP revealed that additional electrostatic complementarity induced by charge transfer or polarization due to complex formation was non-negligible and, therefore, considered important for binding between the proteins. These findings suggest the efficacy of this method for chemical and biological studies.

1. INTRODUCTION

Protein-protein interactions (PPIs) are extensively investigated due to their important roles in numerous biological processes,[1,2] resulting that PPIs are now spotlighted as a new target of drug discovery.[3-8] Additionally, a detailed understanding of PPIs is essential for the design of antibodies used for various research purposes and/or therapeutic applications based on their high affinity and target specificity. Therefore, the importance of computational approaches to provide physicochemical insight into PPIs has increased.

Given that electrostatic interactions are among the most essential components of PPIs, detailed analysis of electrostatic complementarity between proteins is important. The electrostatic potential (ESP) at the PPI interface is generally used to obtain information concerning electrostatic complementarity. Although the ESP of a protein is routinely calculated using a classical force field, an accurate ESP that includes the effect of charge transfer or polarization due to complex formation requires a fully quantum mechanical calculation. However, because of high computational cost of such calculations, few studies[9-11] have attempted ESP calculation for a large molecule like a protein using *ab initio* quantum chemical methods.

Fragment molecular orbital (FMO) method has been applied to reduce the computational effort of *ab initio* quantum chemical calculation of a large molecule.[12,13] In this method, a target molecule is divided into small fragments, and only calculations of monomers and dimers for each fragment are required in order to reduce the computational effort while maintaining chemical accuracy. Another advantage of FMO method is its clear definition of inter-fragment interaction energy (IFIE),[14] which provides detailed information about intramolecular and/or intermolecular interactions. Therefore, FMO method is also known to be a powerful tool for analyzing molecular interactions for large molecules including proteins. This method has been extended to several electron correlation methods, including second- and third-order Møller-Plesset

perturbation theories (MP2 and MP3),[15-21] resolution of the identity (RI) approximation for MP2 and MP3,[22-25] local MP2,[26,27] and coupled cluster theory.[28,29]Several studies in which a fully quantum mechanical ESP was calculated using the FMO method were reported.[30-34] For example, ESP obtained by the FMO method was used to improve determination of atomic charges in classical force fields.[30-32]Ishikawa reported FMO-based calculations of electrostatic properties, including electron density (EDN), ESP, and electric field, at Hartree-Fock and MP2 levels of theory.[33] In this study, a fully quantum mechanical ESP of prion protein (103 amino acid residues) and human immunodeficiency virus type 1 protease (198 amino acid residues) were calculated as illustrative examples, which demonstrated a sufficiently small error associated with fragmentation of the FMO method. Recently, FMO calculations of EDN and ESP were carried out in solution condition using the polarizable continuum model,[34] by which the solvent effect on molecular ESP was detailed investigated. These findings suggest that a fully quantum mechanical ESP can provide reliable information concerning the electrostatic properties of proteins, making it potentially useful for various types of research, including drug discovery. However, to the best my knowledge, computational analysis of the electrostatic complementarity of PPIs using a fully quantum mechanical ESP obtained from the FMO method has not been reported. In this study, a new method for visualizing the electrostatic complementarity of a PPI using a fully quantum mechanical EDN and ESP based on the FMO method is described. To demonstrate the efficacy of this method, the complex of programmed cell death-1 (PD-1) and its ligand (PD-L1), which are important proteins in immunotherapy of cancer,[35,36] was selected as an illustrative example. A recent FMO study by Lim *et al.*[37] analyzed this complex, as well as the antibodies targeting each respective protein, with energetics information rather than the EDN and ESP. The remainder of the paper is presented, as follow. In the next section, theoretical aspects of the method are provided together with implementation and computational details. In the third section, results of the calculations are discussed, and the efficacy of the method is demonstrated.

2. METHOD

2.1. Definition of partial EDN

Here, we consider a complex comprising proteins A and B. In the FMO method, the total EDN of this complex at a position $\mathbf{r}$ is evaluated by the monomer and dimer calculations of the fragments, as follow:

$$\rho_{\mathrm{AB}}(\mathbf{r}) = \sum_I \rho^I(\mathbf{r}) + \sum_{I<J} \rho^{\mathrm{IJ}}(\mathbf{r}), \quad (1)$$

where I and J are indexes of the fragment. The first term is the direct contribution to the total EDN from the monomers, and the second term is a two-body correction from the dimers. The more detailed information about this equation can be found in the previous paper.[33]

We introduce the partial EDN (pEDN) of proteins A and B in the complex condition:

$$\rho_{\mathrm{AB}}^{part-A}(\mathbf{r}) = \sum_{I\in A} \rho^I(\mathbf{r}) + \sum_{I<J\in A} \rho^{\mathrm{IJ}}(\mathbf{r}) + \tfrac{1}{2}\sum_{I\in A}\sum_{J\in B} \rho^{\mathrm{IJ}}(\mathbf{r}), \quad (2)$$

$$\rho_{\mathrm{AB}}^{part-B}(\mathbf{r}) = \sum_{I\in B} \rho^I(\mathbf{r}) + \sum_{I<J\in B} \rho^{\mathrm{IJ}}(\mathbf{r}) + \tfrac{1}{2}\sum_{I\in A}\sum_{J\in B} \rho^{\mathrm{IJ}}(\mathbf{r}). \quad (3)$$

In the case of the pEDN for protein A (equation (2)), the first term is the direct contribution from the monomers of protein A, and the second term is a two-body correction from the dimers of protein A. The third term is a two-body correction from the dimers between proteins A and B, which is halved and added to the pEDN of protein A. For protein B, the pEDN is similarly defined (equation (3)). Notably, the total EDN of the complex (equation (1)) is given by a simple sum of the pEDNs for proteins A (equation (2)) and B (equation (3)):

$$\rho_{\mathrm{AB}}(\mathbf{r}) = \rho_{\mathrm{AB}}^{part-A}(\mathbf{r}) + \rho_{\mathrm{AB}}^{part-B}(\mathbf{r}). \quad (4)$$

As shown in these equations, an advantage of the FMO method is that a partial value can be easily defined by limiting the summation of the equation of the corresponding total value for various physical quantities. For example, the partial energy gradient was defined in a similar fashion, by which geometry optimization of an important site in a protein was efficiently performed in a previous study.[38]

2.2. Definition of partial ESP

In analog with the pEDN, the partial ESP (pESP) of proteins A and B can be defined. In the FMO method, the total ESP at a position$\mathbf{r}$ is evaluated according to the following equation:

$$\phi_{\mathrm{AB}}(\mathbf{r}) = \sum_I \phi^I(\mathbf{r}) + \sum_{I<J} \phi^{\mathrm{IJ}}(\mathbf{r}) + \sum_\alpha \frac{Z_\alpha}{|\mathbf{r}-\mathbf{R}_\alpha|}, \quad (5)$$

where α is the index of an atom, $\mathbf{R}_\alpha$ is the position of the atom, and Z_α is the nuclear charge of the atom. The first term is the direct contribution to the total ESP from the monomers, the second term is a two-body correction from the dimers, and the third term is the ESP due to the atomic nuclei. The more detailed information about this equation can be found in the previous paper.[33]

The pESP can be introduced similarly to the pEDN, i.e.,

$$\phi_{\mathrm{AB}}^{part-A}(\mathbf{r}) = \sum_{I\in A} \phi^I(\mathbf{r}) + \sum_{I<J\in A} \phi^{\mathrm{IJ}}(\mathbf{r}) + \sum_{\alpha\in A} \frac{Z_\alpha}{|\mathbf{r}-\mathbf{R}_\alpha|} + \frac{1}{2}\sum_{I\in A}\sum_{J\in B} \phi^{\mathrm{IJ}}(\mathbf{r}), \quad (6)$$

$$\phi_{\mathrm{AB}}^{part-B}(\mathbf{r}) = \sum_{I\in B} \phi^I(\mathbf{r}) + \sum_{I<J\in B} \phi^{\mathrm{IJ}}(\mathbf{r}) + \sum_{\alpha\in B} \frac{Z_\alpha}{|\mathbf{r}-\mathbf{R}_\alpha|} + \frac{1}{2}\sum_{I\in A}\sum_{J\in B} \phi^{\mathrm{IJ}}(\mathbf{r}). \quad (7)$$

The first and second terms are ESP contributions from the monomers and dimers in protein A or B, respectively, and the third term is the ESP associated with the atomic nuclei in protein A or B. The fourth term is a two-body correction from the dimers formed by proteins A and B, which is halved and added to the pESP. Notably, a simple sum of the pESPs of proteins A (equation (6)) and B (equation (7)) is exactly equivalent to the total ESP of the complex (equation (5)):

$$\phi_{\mathrm{AB}}(\mathbf{r}) = \phi_{\mathrm{AB}}^{part-A}(\mathbf{r}) + \phi_{\mathrm{AB}}^{part-B}(\mathbf{r}). \quad (8)$$

2.3. Definition of the PPI interface using the pEDN

The pEDN is then used to define the PPI interface in the complex. A surface formed by the positions where the pEDNs of proteins A and B are same value is naturally considered as the interface between the proteins. That is, the PPI interface can be defined by positions$\mathbf{r}$ satisfying the following equation:

$$\rho_{\mathrm{AB}}^{part-A}(\mathbf{r}) - \rho_{\mathrm{AB}}^{part-B}(\mathbf{r}) = 0. \quad (9)$$

The left side of equation (9) is subtraction of the pEDNs of proteins A and B. Thus, in other words, a surface with a zero value of the differential EDN of the pEDNs is defined as the PPI interface. This definition method is more consistent with chemical intuition compared with the other methods, e.g., a definition method using atomic distance between proteins.

2.4. Analysis of electrostatic complementarity using the pESP

By comparison between the pESPs of proteins A and B at the PPI interface, the electrostatic complementarity of the two proteins in the complex condition can be analyzed. The most important point of this analysis is that a fully quantum mechanical EDN and ESP are used, by which the effect of charge transfer or polarization due to complex formation is fairly considered. Generally, quantum chemical calculations of the EDN and

ESP of a large molecule like a protein are difficult because too much computational effort is required. By using the FMO method, however, the computational effort can be greatly reduced.

2.5. Implementation

In this study, the pEDN (equations (2) and (3)) and pESP (equations (6) and (7)) were implemented in PAICS,[39] which is our original FMO program package. In PAICS, EDN and ESP at the MP2 level of theory can be efficiently calculated by using RI approximation.[24] In the current implementation, the grid data of the pEDN and pESP of proteins A and B are transferred to separate files, and the differential EDN is obtained by simply subtracting the pEDNs and used to define the PPI interface. To analyze the electrostatic complementarity of the PPI, the pESPs of proteins A and B are visualized at the PPI interface. Here, such a visualization of the grid data was performed with UCSF Chimera.[40]

2.6. Computational details

To show a potential of this method, the interaction of PD-1 and PD-L1, which is one of the most important PPIs in immunotherapy of cancer,[35,36] was selected as an illustrative example. In this study, the X-ray structure of the PD-1/PD-L1 complex was downloaded from the PDB (PDB-ID: 4ZQK).[41] The structure contains the amino acid residues ranging from D33 to E84 and from S93 to E146 for PD-1 and from A18 to A132 for PD-L1. Missing hydrogen atoms were added to the structure, and N- and C-terminals of these peptide chains were capped with -$COCH_3$ and -$NHCH_3$, respectively. Energy minimization was performed with the AMBER99SB[42] force field using the AMBER 10 package.[43] As a result, the net charges of PD-1 and PD-L1 of the model used here were +2 and -1, respectively. To examine the effect of net charge, a neutralized model of the complex was prepared by adding two chloride ions near the positively charged residues of PD-1 (R112 and R143) as counter ions and one sodium ion near the negatively charged residue of PD-L1 (D90). These residues were sufficiently distant from the PPI interface. After adding these ions, energy minimization was then performed.

As mentioned above, pEDN and pESP were calculated as grid data in the current implementation. Here, the grid with a separation of 0.3 Å which covered the interface between PD-1 and PD-L1 was used (total number of grid points: 712,659). The pEDN and pESP at the grid points were calculated at the MP2 level of theory using RI approximation, where cc-pVDZ basis sets[44] and the auxiliary basis sets produced by Weigend et al.[45] were used. For the FMO calculations, each amino acid residue was treated as a single fragment, except for cysteines involved in a disulfide bond, which were merged into one fragment. In addition to calculations for the complex, FMO calculations for isolated PD-1 and PD-L1 were performed using the same atomic coordinates. By comparison of ESPs calculated in the isolated condition with those calculated in the complex condition, the effect of charge transfer or polarization due to complex formation can be evaluated.

3. RESULTS AND DISCUSSION

3.1 Definition of the PPI interface

The pEDNs of PD-1 and PD-L1 were obtained from the FMO calculation of the complex using the equation (2) or (3), from which the differential EDN was also obtained by simply subtracting them. In Figure 1-A, the surface with a zero value of this differential EDN is pictured (the pEDNs of PD-1 and PD-L1 on this surface are the same value). As mentioned above, this surface is naturally considered as the PPI interface. In Figure 1-B, the pEDN are visualized on this surface. We note that positions with a pEDN larger than 0.001 a.u. (colored region) are widespread on the PPI interface, indicating that the EDNs of PD-1 and PD-L1 widely overlap with each other. Positions where the pEDN is larger than 0.01 a.u. (blue or purple) mean to be a significantly large overlap of the EDNs caused by close contacts between amino acid residues of the two proteins. For example, position $\mathbf{P_1}$in Figure 1-B is caused by contact between K131 of PD-1 and Q66 of PD-L1 (distance: 1.74 Å). Similarly, position $\mathbf{P_2}$ is cause by contact between Y68 of PD-1 and D122 of PD-L1 (distance: 1.61 Å). At the other positions with large pEDN values, amino acid residues are also in close contact.

These results suggest that an undulating PPI interface involving numerous amino acid residues was reasonably determined according to their pEDNs. Additionally, by visualizing the value of the pEDN at the PPI interface, the range of atomic contacts between proteins was clearly represented.

3.2 Visualization of electrostatic complementarity

The pESPs of PD-1 and PD-L1 were obtained from FMO calculation of the complex using equation (6) or (7). In this paper, they are denoted as$\phi_{\rm com}^{PD-1}(\mathbf{r})$ and$\phi_{\rm com}^{PD-L1}(\mathbf{r})$, respectively. The subscript "com" is used to explicitly state that they were calculated in the complex condition. The values of$\phi_{\rm com}^{PD-1}(\mathbf{r})$ and$\phi_{\rm com}^{PD-L1}(\mathbf{r})$ at the PPI interface are visualized in Figure 2-A and B, respectively. The positions with positive (blue) and negative (red) values are scattered along the PPI interface, indicating that both PD-1 and PD-L1 form a complicated ESP map at the interface. A more interesting point is that electrostatic complementarity of PD-1 and PD-L1 is clearly shown by comparison between these pESP maps. For example, pESP values for PD-1 and PD-L1 at position$\mathbf{P_3}$ are positive and negative, respectively, indicating that attractive electrostatic interaction exists around this position. Similar observations are made for other positions ($\mathbf{P_4}$, $\mathbf{P_5}$,$\mathbf{P_6}$, and $\mathbf{P_7}$). By this analysis, we can understand that a high degree of electrostatic complementarity exists between PD-1 and PD-L1 at the PPI interface.

We also note that the area with a positive value is larger than that with a negative value in the pESP map for PD-1 (Figure 2-A). Conversely, the area with a negative value is larger than that with a positive value for PD-L1 (Figure 2-B). As mentioned above, the net charges of PD-1 and PD-L1 of the structure model used here were +2 and -1, respectively. Therefore, it is assumed that the pESPs shifted to positive or negative in whole. Such an overall shift just depends on the structure modeling. For example, D33-E84 and S93-E146 of PD-1 were included in the complex model, which just depended on the X-ray structure used for the modeling. As a result, the net charge of PD-1 was +2, causing the overall shift in the pESP. To examine the effect of net charges, another complex model was prepared with the net charges neutralized. The pESP maps of the neutralized PD-1 and PD-L1 are given in Figure S1-A and B, respectively. We note that a green area, where the pESP value is around zero, is larger than that of Figure 2-A and B. This result shows that the overall shift in pESP due to the net charge was removed by neutralization. Effect of the net charge is considered to be limited in the overall shift because similar discussion about the electrostatic complementarity is led from both the neutralized and not neutralized models.

In this study, all the FMO calculations were performed under vacuum conditions, i.e., no solvent effect was considered. Recently, the solvent effect on EDN and ESP obtained from FMO calculations was investigated using the polarizable continuum model.[34]It was reported that solvent made a large contribution to ESP at the molecular surface while the effect on EDN was sufficiently small. The solvent effect on ESP at the PPI interface is expected to be lower than that at the molecular surface, because generally solvent molecules do not directly contact the PPI interface. To evaluate the solvent effect on electrostatic complimentarily, the definition of the pESP given in equations (6) and (7) should be modified to include the solvent molecules as the third part of the system. This is an important and interesting extension of this method, which should be addressed in a future study.

3.3 Effect of complex formation

The ESPs of PD-1 and PD-L1 in the isolated condition were calculated to examine the effect of complex formation. In this paper, the ESPs of the isolated PD-1 and PD-L1 are denoted as$\phi_{\rm iso}^{PD-1}(\mathbf{r})$ and$\phi_{\rm iso}^{PD-L1}(\mathbf{r})$, respectively. The values of the $\phi_{\rm iso}^{PD-1}(\mathbf{r})$ and$\phi_{\rm iso}^{PD-L1}(\mathbf{r})$ at the PPI interface are visualized in Figure 2-C and D, respectively. Although they are similar to $\phi_{\rm com}^{PD-1}(\mathbf{r})$ and$\phi_{\rm com}^{PDL-1}(\mathbf{r})$ (Figure 2-A and B), there is noted to be a slight difference. This difference is considered to be caused by charge transfer or polarization due to complex formation.

To clarify the effect of complex formation, the map of the differential ESP between $\phi_{\rm com}^{PD-1}(\mathbf{r})$ and$\phi_{\rm iso}^{PD-1}(\mathbf{r})$ or$\phi_{\rm com}^{PD-L1}(\mathbf{r})$ and$\phi_{\rm iso}^{PD-L1}(\mathbf{r})$ is shown in Figure 2-E or F, where the value range is one-tenth that of the other ESP maps. We note that the amplitude of the change in ESP induced by complex formation is more than 10% of that of the ESP value, indicating that the effect of charge transfer or polarization due to complex

formation is not negligible. Additionally, the amplitude of the positive or negative value is enhanced by complex formation. For example, the value of the differential ESP of PD-1 around position $\mathbf{P_3}$ is positive (Figure 2-E), and the value of$\phi_{\mathrm{iso}}^{PD-1}(\mathbf{r})$ around position$\mathbf{P_3}$ is also positive (Figure 2-C), suggesting that amplitude of the positive ESP value for PD-1 at this position increases due to complex formation. On the other hand, the amplitude of the negative value of PD-L1 around position $\mathbf{P_3}$is also enhanced by complex formation. This indicates that the degree of electrostatic complementarity around position$\mathbf{P}_3$ increases due to complex formation. A similar situation is observed at the other positions ($\mathbf{P_4}$, $\mathbf{P_5}$,$\mathbf{P_6}$, and $\mathbf{P_7}$). These results clearly show that additional electrostatic complementarity is induced by charge transfer or polarization due to complex formation, and that its amplitude is not negligible (more than 10% of the original ESP value). Consequently, we can consider this induced electrostatic complementarity as an important factor for binding between PD-1 and PD-L1. Moreover, this argument is equally valid when using the ESP maps calculated with the neutralized models (see Figure S1-C, D, E, and F). Therefore, our conclusion about the induced electrostatic complementarity is largely independent of the models. As demonstrated by this illustrative example, the method visualizing the pESP is a promising tool for analyzing electrostatic complementarity of PPIs.

3.4 Analysis of the PPI together with IFIE

Although electrostatic interaction is an essential component of the molecular interaction, non-electrostatic interaction, i.e., dispersion interaction or van der Waals interaction, is equally important. For fairly understanding of PPI, therefore, non-electrostatic interaction should be considered. As mentioned above, FMO calculations provide the IFIE, that is known to be useful for analyzing the molecular interaction, including the non-electrostatic interaction.

In Figure 3, the RI-MP2 correlation contributions to the interaction energy (i.e., the non-electrostatic interaction energy) are given for amino acid residues in PD-1 and PD-L1 located at the PPI interface. These values were obtained by simply summing up the RI-MP2 correlation energies of the IFIE. The location of these amino acid residues is also given in Figure 3, together with amplitude of the correlation contributions represented by the depth of color. By this analysis, we can understand which residues at the PPI interface are importantly undertaken the non-electrostatic interaction with the other protein. For example, Q75 and I134 of PD-1 have a large non-electrostatic interaction with PD-L1, and Y123 of PD-L1 has an especially large non-electrostatic interaction with the PD-l.

As shown here, information about the non-electrostatic interaction of PPI can be obtained by analysis using the electron correlation contribution of the IFIE. Thus, the combination of the visualization method for electrostatic complementarity and IFIE analysis is one of potential choices to provide physicochemical insight into a PPI.

4. CONCLUSIONS

In this study, a novel method for visualizing the electrostatic complementarity in a PPI was proposed using the pEDN and pESP, which were introduced by limiting the summation of the FMO equations of the total EDN and ESP. In this method, the PPI interface is defined using the pEDNs of the proteins obtained from the FMO calculation for the complex, followed by visualization of the pESP of the proteins at the PPI interface. To show the potential of this method, the PD-1/PD-L1 complex was selected as an illustrative example. The results successfully demonstrated that the PPI interface was appropriately determined according to the pEDN, and that electrostatic complementarity was clearly represented by visualizing the pESP. Interestingly, additional electrostatic complementarity induced by charge transfer or polarization due to complex formation was explicitly revealed, indicating its important role in PD-1/PD-L1 binding. Notably, such information cannot be obtained without a fully quantum mechanical ESP. Thus, we can conclude that the method proposed in this study is useful for chemical and biological investigations.

A potential application of this method is the design of antibodies, which is recently used as a therapeutic agent because of its high affinity and specificity to the target protein. Especially, the specificity is considered to be strongly related to the electrostatic complementarity between antibody and its target. Consequently,

use of the proposed method could increase the efficiency of antibody design and will be the focus of future research.

SUPPORTING INFORMATION

Results of calculations using the charge-neutralized model (Figure S1).

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Notes

The author declares no competing financial interest.

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FIGURE LEGENDS

Figure 1: Surface showing a value of zero for the differential EDN between pEDNs for PD-1 and PD-L1 (A). pEDN values at the surface and examples of pairs of amino acid residues in close proximity (B).

Figure 2: pESP values for PD-1 ($\phi_{\rm com}^{PD-1}(\mathbf{r})$) and PD-L1 ($\phi_{\rm com}^{PD-L1}(\mathbf{r})$) in complex condition at the PPI interface (A and B). ESP values for PD-1 ($\phi_{\rm iso}^{PD-1}(\mathbf{r})$) and PD-L1 ($\phi_{\rm iso}^{PD-L1}(\mathbf{r})$) in isolated condition at the PPI interface (C and D). Differential ESP values between $\phi_{\rm com}^{PD-1}(\mathbf{r})$ and$\phi_{\rm iso}^{PD-1}(\mathbf{r})$ and between$\phi_{\rm com}^{PD-L1}(\mathbf{r})$ and$\phi_{\rm iso}^{PD-L1}(\mathbf{r})$ at the PPI interface (E and F). The value ranges are -0.1 to +0.1 a.u. for A-D, and -0.01 to +0.01 a.u. for E and F.

Figure 3: RI-MP2 correlation contribution to the interaction energy of amino acid residues located at the

PPI interface of PD-1 (A) and PD-L1 (B). The locations of these amino acid residues are also given, with the amplitude of the interaction energy represented by color.