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Research on Repressing Allergen Cry j 1 Released from Japanese Cedar Pollen Using Todomatsu Oil

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Abstract: Japanese cedar (JC, *Cryptomeria japonica*) pollen allergens are the primary cause of JC pollinosis, a widespread seasonal allergic disorder and a significant public health issue in Japan. Nevertheless, rare information on repressing the pollen allergens released from JC pollen is available. This study aims to validate the repression of allergen Cry j 1 (the dominant JC pollen allergen that triggers JC pollinosis) using todomatsu oil produced from *Abies sachalinensis* waste, through surface plasmon resonance (SPR) experiments, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and blind docking simulation. The findings revealed that todomatsu oil reduces the antibody reactivity and content of allergen Cry j 1 by 85.96% and 42.12%, respectively. The docking modeling suggested that alkyl hydrophobic forces, pi–sigma bonding, and hydrogen bonding are the principal motivating forces for todomatsu oil components to dock with allergen Cry j 1. More than 50% of the amino-acid residues docked by each todomatsu oil component (except α-pinene) are hydrophobic. Furthermore, oil components, especially β-maaliene, exhibited potent repression of allergen Cry j 1. Consequently, todomatsu oil, due to its inexpensive, available, convenient, and ecologically beneficial nature, is a viable measure to repress allergen Cry j 1.

Keywords: Japanese cedar (*Cryptomeria japonica*) pollen; aeroallergens; pollinosis (pollen allergy); essential oil; repression; molecular docking

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1. Introduction

Pollinosis (also referred to as pollen allergy), the seasonal allergic rhinitis triggered by pollen allergens [1], is intractable and lowers the quality of life (QOL) as it worsens [1]. Japanese cedar (JC, *Cryptomeria japonica*) pollinosis induced by JC pollen allergens is a widespread seasonal allergic disorder and the chief issue for public health in Japan [2–6]. Although JC pollinosis is viewed as a severe societal problem in Japan, the situation is worsening rather than improving [2]. Its incidence has risen dramatically since the initial report in 1964 [1,3,7,8], reaching 16.2% in 1998, 26.5% in 2008, and 38.8% in 2019 [2,9].

Japanese cedar grows in abundance throughout Japan [2]. It has been demonstrated that both the higher airborne JC pollen counts and the longer exposure duration raise the prevalence [2,10,11]. Other current findings illustrate that early exposure to pollen tremendously boosts the risk of pollinosis occurrence [2,8,9,12]. Preventing pollen exposure on the basis of pollen dispersal information is acknowledged as a nonpharmacological intervention for JC pollinosis in the *Japanese guidelines for allergic rhinitis 2020* [1]. According to recent studies, the prevention of JC pollen inhalation generally acts as a physical barrier to protect sensitive populations from pollen grain inhalation, including air purifiers, pollen screens, car filters, masks and sunglasses, nasal filters, nasal ointments, powders, and oils, nasal irrigation, and artificial tears and eye compresses [1,13–33]. Although nonpharmacological measures without side-effects are of great interest in use to moderate allergy

symptoms and improve the QOL of patients [13], the restrictions are obvious. For example, employment in air purifiers, pollen screens, and car filters is beneficial to prohibit the penetration of pollen particles into indoor places, yet fails when walking outside. Wearing a mask and sunglasses offers shelter for sensitive individuals outside during the pollen dispersal season but is inefficient in a gale [13].

While countless approaches have been issued to prevent JC pollen exposure, knowledge on repelling or lowering the inhalation of pollen allergens emitted from JC pollen is scarce. Moreover, it is thought that plant pollen-derived aeroallergens are the primary airborne allergens contributing to allergic rhinitis [34,35]. According to earlier research, pollen and aeroallergens are accountable for over 92% and almost 63% of allergic rhinitis cases, respectively [34,36,37]. Four allergens (Cry j 1, Cry j 1, Cry j 3, and Cry j 4) have been isolated from JC pollen so far [38–40]. Cry j 1, the first predominant allergen recognized in JC pollen, is homologous to pectate lyase and appears on the small particles on the pollen surface and the outer wall [38,41–43]. Cry j 2, homologous to polymethyl galacturonate, exists in pollen starch granules and pollen membrane [38,41–43]. Cry j 3, analogous to thaumatin-like protein, is a homolog of Jun a 3, a dominant mountain cedar (*Juniperus ashei*) pollen allergen [38,40]. Cry j 4, the latest identified JC pollen allergen, is homologous to Chao 3, a novel crucial allergen of Japanese cypress (*Chamaecyparis obtusa*) pollen [39]. Notably, Cry j 1 and Cry j 2 are the major pollen allergens eliciting JC pollinosis [38,44]. The finding of IgE specific to these two allergens in the serum of more than 90% of patients suffering from JC pollinosis has been reported [3]. Moreover, they were discovered in the atmosphere prior to the onset of pollen dispersion in one investigation [41]. Accordingly, decreasing or eliminating the absorption of main aeroallergens is vital for sufferers of JC pollinosis.

Todomatsu oil, a newly released acclaimed product for avoiding pollen allergens, has been depicted as possessing the capacity to weaken JC pollen allergens as the result of encasing JC pollen (https://morilabo.st-c.co.jp/, accessed on 10 April 2023). It is an essential oil derived from the *Abies sachalinensis* waste, such as bark and leaves, thus being inexpensive, available, and ecologically beneficial [45]. Furthermore, its various types (stick, pot, spray, and sticker types) satisfy indoor and outdoor defense (https://www.st-c.co.jp/news/newsrelease/2021/20211215_001708.html, accessed on 10 April 2023). For instance, the pot type is appropriate for interior defense, the stick type is suitable for exterior protection, usually applied on the mask, and the sticker type is reasonable for kids who do not want to wear a mask. Hence, it is a possible prospective nonpharmacological measure for JC pollinosis prevention since it compensates for most drawbacks of other treatments. It has been published as an inhibitor of house dust mite allergens Der f 1, Der p 1, and Der f 2 [46,47]. Nevertheless, its potential to repress the JC pollen allergens has not been validated or published.

This research aimed to ascertain the capability of todomatsu oil to repress JC pollen allergens according to its effects on the antibody reactivity and content of allergen Cry j 1, the most ubiquitous JC pollen allergen. Cry j 1, located on the pollen surface, is the major ingredient of JC pollen extract [38]. In addition, it possesses the highest IgE-binding prevalence in patients suffering from JC pollinosis [38]. Moreover, a 4 year monitoring record revealed that pollinosis symptoms of patients appeared when the atmospheric level of allergen Cry j 1 was at 1–3 pg/m^3 and aggravated when it abruptly elevated [48]. Therefore, the inhibition of allergen Cry j 1 by todomatsu oil was validated first. Herein, the repression of the antibody-binding activity of Cry j 1 by todomatsu oil was assessed using a surface plasmon resonance (SPR) experiment. The antibody used in this assay was monoclonal antibody (mAb) 013, which has been proven to recognize the foremost allergenic epitope on Cry j 1, closely linked with IgE recognition [49]. Allergen Cry j 1, a glycoprotein homologous to pectate lyase [2,3,50,51], appears as two bands on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), most likely owing to its glycosylation heterogeneity. Furthermore, an automated docking simulation was applied

to predict the docking modes and inhibitory potency of each todomatsu oil component toward the target allergen Cry j 1.

2. Materials and Methods

2.1. Sample Preparation

Tomizawa et al. (2022) reported that the Cry j 1 concentration in collected atmosphere samples is about 0–650 ng/mL during the pollen season [41]. For the SPR experiment, at least five concentrations of samples are required for kinetics measurement, and the detection limit is 10 pg/mL [52]. As a result, standardized allergen Cry j 1 (Funakoshi Co., Ltd., Bunkyoku, Tokyo, Japan) was diluted to 500, 250, 125, 62.5, and 31.25 ng/mL using HBS-EP buffer (general purpose buffer, Cytiva Co., Ltd., Shinjuku, Tokyo, Japan) for SPR experiments. SDS-PAGE using Coomassie blue staining has a detection limit of about 0.1–0.5 μg protein [53]. The maximum pore volume of each lane in the gel is 20 μL. Accordingly, 0.8 μg (8 μL, 100 μg/mL) of allergen Cry j 1 diluted by PBS (pH 7.4) was chosen as the initial concentration for SDS-PAGE. The diluted allergen Cry j 1 solution mixed with todomatsu oil (Aroma Laboratory Co., Ltd., Shinjuku, Tokyo, Japan) in a ratio of 9:1 (*v*/*v*) stood at room temperature for 1 h as the test group. The todomatsu oil used in the experiments was crude oil extracted from *Abies sachalinensis*. Under an identical set of conditions, the oil-free duplicate was set as the control group.

2.2. Surface Plasmon Resonance Experiments for In Vitro Antibody Reactivity Studies

Kinetics measurement by SPR experiment is acknowledged as the gold standard for determining binding activity [54]. Kinetics measurement on the CM5 sensor chip (Cytiva Co., Ltd., Shinjuku, Tokyo, Japan) was achieved at 25 °C utilizing the single-cycle kinetics (SCK) method in the Biacore X100 device (Cytiva Co., Ltd., Shinjuku, Tokyo, Japan) for antibody reactivity studies of Cry j 1. Anti-Cry j 1 mAb 013 (BioDynamics Laboratory Co., Ltd., Bunkyoku, Tokyo, Japan) in 10 mM acetate buffer (pH 5.0) (Cytiva Co., Ltd., Shinjuku, Tokyo, Japan) was anchored on a CM5 sensor chip by amine coupling before the determination. The analyte (Cry j 1) with five concentrations prepared as described in Section 2.1 was then injected onto the functionalized surface at increasing concentrations without regeneration between injections. Regeneration was performed with 10 mM glycine pH 3.0 (Cytiva Co., Ltd., Shinjuku, Tokyo, Japan) by a 1 min pulse, following the kinetics measurement. The association (K_a) and dissociation (K_d) rate constants, and the dissociation equilibrium constant (K_D) were received from the direct curve fitting of the sensorgrams to a Langmuir 1:1 model of interaction. The binding activity of the antigen to its specific antibody was assessed on the basis of the K_D value. A higher K_D value denotes a lower binding activity. The lowering extent was estimated as an inhibitory rate using Equation (1).

$$Inhibitory\ rate\ (\%) = \frac{V_T - V_C}{V_T} \quad (1)$$

where V_T and V_C are the K_D values of the test and control groups, respectively.

2.3. Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis Evaluating Allergen Content

SDS-PAGE was chosen for allergen Cry j 1 content analysis. The pretreated samples stated in Section 2.1 were combined with 6× loading buffer (Geno Technology Inc., St. Louis, MO, USA) and denatured at 99 °C. Each allergen sample was then loaded into a unique lane in 12.5% polyacrylamide gel (ATTO Co., Ltd., Chuo, Tokyo, Japan) and electrophoresed for 90 min at a constant 250 V. The gel was subsequently colored with Coomassie brilliant blue R-250 (ATTO Co., Ltd., Chuo, Tokyo, Japan) and decolorized with deionized water. Image J 1.48v (National Institutes of Health, Bethesda, MD, USA) was employed to measure the gray value of the band. The decline rate of allergen content was calculated using Equation (2).

$$Decline\ rate\ (\%) = \frac{B_T - B_C}{B_T} \quad (2)$$

where B_T and B_C are the gray values of the bands for the test and control groups, respectively.

2.4. Blind Molecular Docking Approach Modeling the Docking Modes

An automated docking simulation, widely applied during the computer-aided drug design process [55], was achieved with PyRx software 0.8v (San Diego, CA, USA) to disclose the docking modes of todomatsu oil components to the target allergen Cry j 1. The todomatsu oil components (Table A1 in Appendix A), comprising bornyl acetate (25.75%), camphene (20.25%), α-pinene (18.25%), β-phellandrene (12.05%), β-pinene (7.55%), limonene (5.75%), β-myrcene (4.45%), tricyclene (2.25%), β-maaliene (1.25%), borneol (1.00%), α-terpinolene (0.95%), and 3-carene (0.50%), were supplied by Japan Aroma Laboratory Co., Ltd. [45–47]. Their structures were obtained in SDF format from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/, accessed on 28 December 2022) and converted to PDBQT format in Open Babel of PyRx [46,47,55–58]. The predicted 3D structure of allergen Cry j 1 (AlphaFold DB: P18632) was retrieved simultaneously from the AlphaFold Protein Structure Database (https://alphafold.ebi.ac.uk/, accessed on 28 December 2022) (Figure 1) and transformed to a macromolecular form in PyRx [46,47,55–58]. Subsequently, the entirely automated docking simulation was performed in AutoDock Vina of PyRx [46,47,55,59–61]. The center grid box was set to the dimensions 64.6450 × 50.9387 × 63.6033 Å. The docking modes and docking energy (ΔG) are the foundational findings in the docking procedure. The oil component-Cry j 1 complexes and docked amino-acid residues of the target allergen were visualized with Biovia Discovery Studio 4.5 (BIOVIA 2020) [46,47,62–64]. The docking score and inhibition constant (*Ki*) of each oil component to allergen Cry j 1 were calculated as a function of docking energy using the following equations [46]:

$$Docking\ score = -\Delta G \tag{3}$$

$$Ki = e^{\frac{\Delta G \times 1000}{Rcal \times TK}} \tag{4}$$

where Δ*G* is the docking energy, *Rcal* is 1.987, and *TK* is 298.15 [46,65–67]. A higher docking score indicates tighter adhesion of the oil component to the allergen Cry j 1. *Ki* value, the inhibitor dose necessary to cause half-maximum repression, represents the inhibitory effect of the oil component on the allergen [46,62,65,68]. A lower *Ki* value denotes a more powerful repression of oil components toward the allergen.

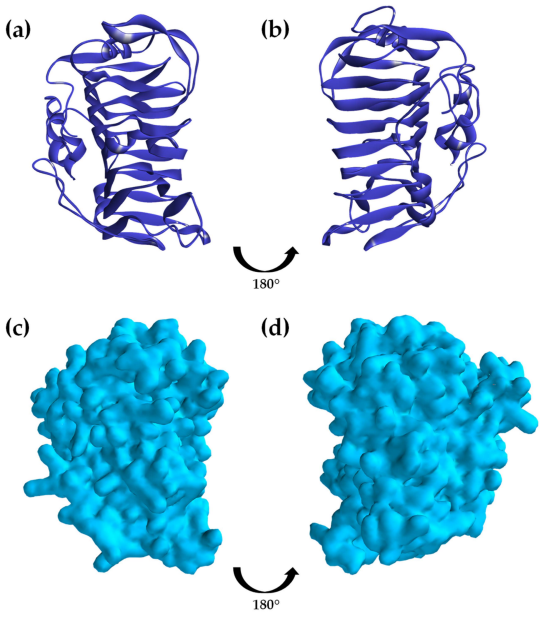

Figure 1. Model structure of allergen Cry j 1 (P18632) shown in two orientations with ribbon (**a**,**b**) and molecular surface (**c**,**d**) representations.

2.5. Statistical Analysis

All experiments were conducted in triplicate. OriginLab Pro 8 was employed to analyze and plot the data. One-Way ANOVA was conducted using SPSS version 18 (SPSS Inc., Chicago, IL, USA) to examine differences between groups. Statistical significance was determined at a threshold of $p < 0.05$.

3. Results

3.1. Antibody Reactivity of Cry j 1 Reduction via Todomatsu Oil

To assess the potentiality of todomatsu oil in repressing the antibody reactivity of Cry j 1, its ability to repress the reactivity of Cry j 1 with its specific mAb 013 was estimated through SPR experiments. As shown in Table 1, todomatsu oil significantly inhibited the binding activity of Cry j 1 to mAb 013, with an inhibitory rate of 85.96%. Consequently, todomatsu oil effectively repressed the antibody reactivity of allergen Cry j 1.

Table 1. The dissociation constants (K_D) of allergen Cry j 1 in test and control groups.

Samples	Test Group	Control Group
K_D (M)	4.03×10^{-9} *	5.66×10^{-10}
Inhibitory rate (%)	85.96	

A higher K_D value denotes a weaker antibody binding activity of the allergen. Test and control groups: allergen Cry j 1 mixed with todomatsu oil and the oil-free duplicate, respectively. * Significant difference ($p < 0.05$).

3.2. Cry j 1 Content Dilution by Todomatsu Oil

The capacity of todomatsu oil for the repression of allergen Cry j 1 content was evaluated using SDS-PAGE. The results illuminated a significant decline in the content of allergen Cry j 1 by 42.12% (Figure 2). This indicates that todomatsu oil also lowered the Cry j 1 content. Therefore, todomatsu oil is a promising approach to protect the pollen-vulnerable population from Cry j 1 exposure, the most prevalent JC pollen allergen.

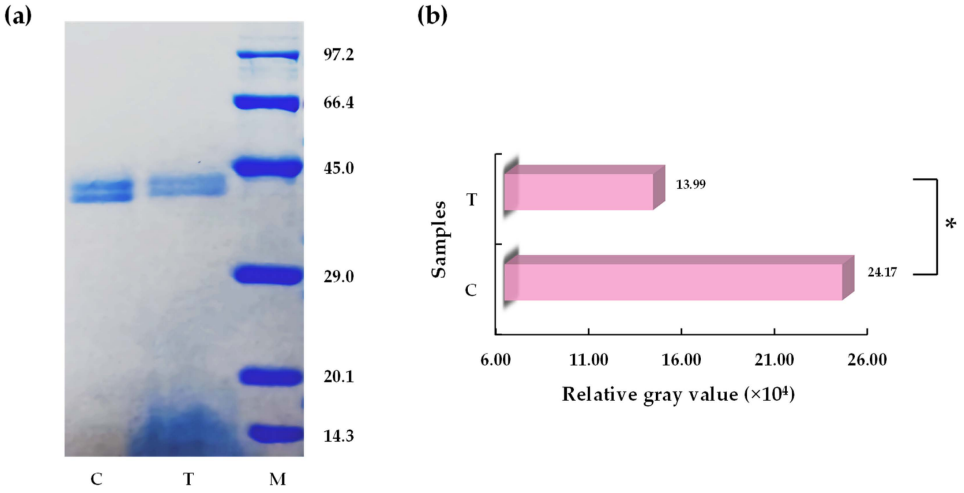

Figure 2. Variation of Cry j 1 content in the test and control groups: (**a**) determination of Cry j 1 content using SDS-PAGE; (**b**) evaluation of relative gray values of the bands in the SDS-PAGE results. Test and control groups: allergen Cry j 1 mixed with todomatsu oil and the oil-free duplicate, respectively. M: marker. * Significant difference ($p < 0.05$).

3.3. Docking Modes of Todomatsu Oil Components to Cry j 1

An automated docking simulation is a typical technique to forecast the docking modes of ligands to macromolecules. Alkyl hydrophobic forces, pi–sigma bonding, and hydrogen bonding are the primary motivating forces behind the docking of todomatsu oil components to allergen Cry j 1 according to the docking model (Figure 3).

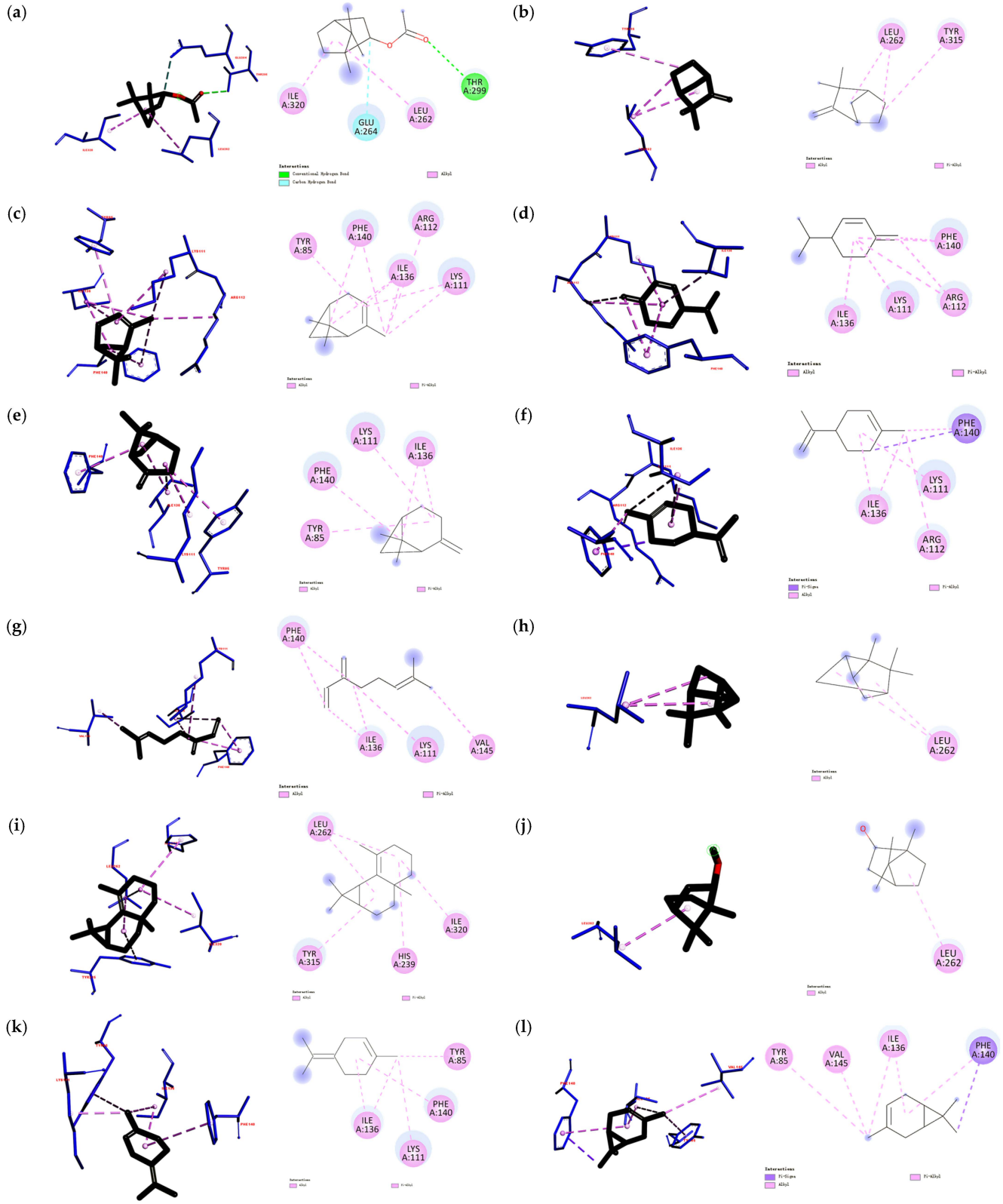

Figure 3. Docking modes of todomatsu oil components to allergen Cry j 1 presented as 3D and 2D diagrams. (**a**) Bornyl acetate, (**b**) Camphene, (**c**) α-Pinene, (**d**) β-Phellandrene, (**e**) β-Pinene, (**f**) Limonene, (**g**) β-Myrcene, (**h**) Tricyclene, (**i**) β-Maaliene, (**j**) Borneol, (**k**) α-Terpinolene, (**l**) 3-Carene.

The formation of covalent bonds, the most formidable natural bonding [69], frequently blocks the activity of the biological target due to its irreversible property and high surface

coverage after formation [70]. Accordingly, the pi–sigma bonding between todomatsu oil components and allergen Cry j 1 possibly breaks or impairs the bioactivity of Cry j 1 (Figure 3 and Table A1).

More than half of the amino-acid residues docked by each todomatsu oil component (excluding α-pinene) are hydrophobic, particularly borneol and tricyclene, merely docking to hydrophobic amino acids (Figure 3 and Table A1). To our knowledge, a hydrophobic core in the center of a protein acts as the fundamental factor maintaining its stability and bioactivity [69–71]. Accordingly, the hydrophobic forces present in todomatsu oil components and allergen Cry j 1 potentially serve as considerable motivation to vary the protein conformations and folding behavior, resulting in the blockade of mAb 013 binding to Cry j 1, owing to the cardinal allergic epitopes on Cry j 1 being conformational epitopes [49,72]. Furthermore, this possibly gives rise to the faster degradation of Cry j 1, leading to a reduction in allergen level.

3.4. Inhibitory Potency of Todomatsu Oil Components toward Cry j 1

The docking score and inhibition constant, independently reflecting the adhesion and inhibitory potency of todomatsu oil components toward allergen Cry j 1 [46,62,65,68], are displayed in Figure 4a and Table A1. A higher docking score suggests a stronger docking of oil component to the allergen Cry j 1. Contrarily, a lower *Ki* value signifies a more potent repression of the allergen. According to the results in Figure 4b, the *Ki* value presented a moderate negative linear correlation with the docking score (R^2 = 0.78). In other words, oil components with a higher docking score possessed a smaller *Ki* value. As a result, the better inhibitory potency of a component toward allergen Cry j 1 was represented as a higher docking score or a lower *Ki* value. The findings of docking simulation imply that oil components displayed potent inhibition of allergen Cry j 1, especially β-maaliene, α-terpinolene, and 3-carene, followed by limonene, α-pinene, β-phellandrene, β-pinene, bornyl acetate, and camphene with a moderate inhibitory potency.

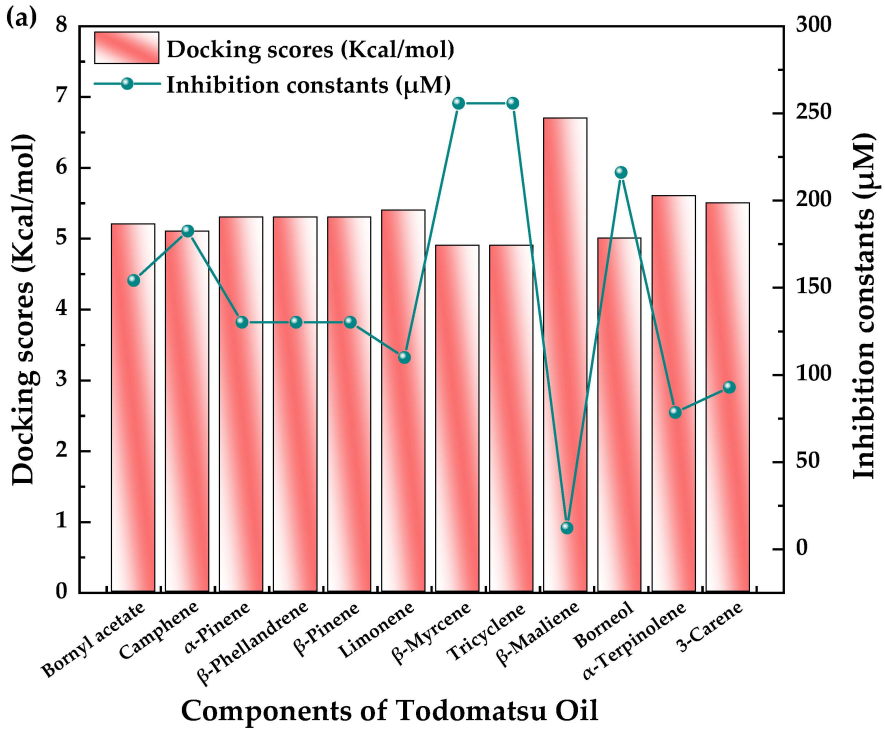

Figure 4. *Cont.*

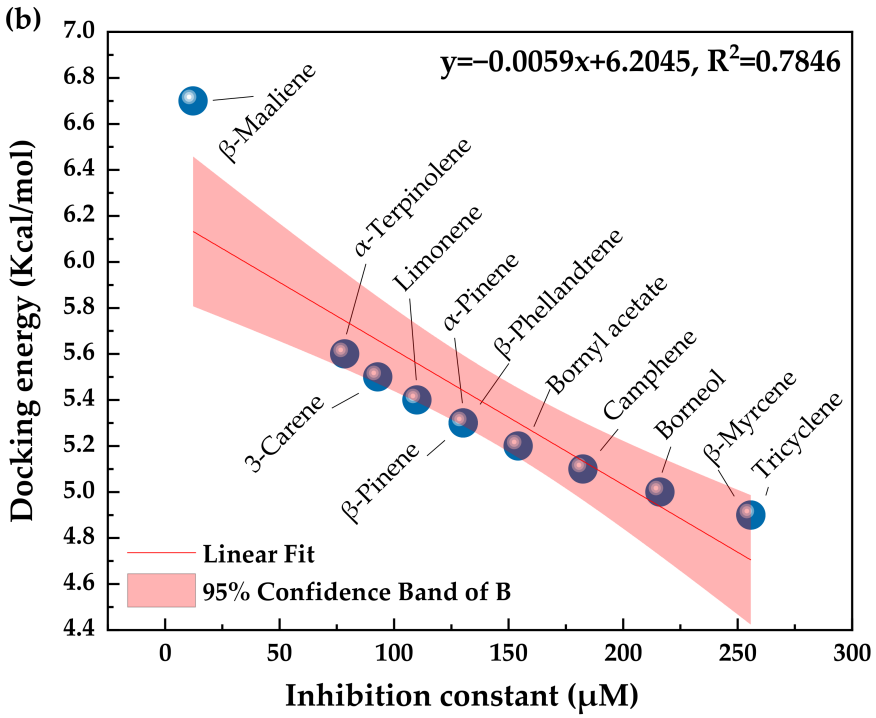

Figure 4. Inhibitory potency of todomatsu oil components toward Cry j 1. Docking score and inhibition constant of each oil component to Cry j 1 (**a**) and their regression analysis (**b**).

4. Discussion

Despite being the primary airborne allergens and an essential trigger of allergic rhinitis, airborne allergens originating from plant pollen still receive little attention when trying to reduce exposure. This research unveiled that todomatsu oil is a promising novel strategy for repressing the JC pollen allergen Cry j 1, which is the primary trigger for JC pollinosis. Todomatsu oil showed inhibitory ability toward allergen Cry j 1 by reducing its antibody reactivity and content. The docking simulation outcomes showed that the hydrophobic amino acids on Cry j 1 are the preferred docking amino acids for todomatsu oil components (Figure 3 and Table A1). As we know, a hydrophobic center in the protein functions as the essential portion preserving its stability and bioactivity [69–71]. As a result, the hydrophobic forces that exist in todomatsu oil components and allergen Cry j 1 possibly alter the protein conformations and folding behavior, which is potentially responsible for the reductions in antibody reactivity and content. The mAb 013 recognizes the significant allergenic epitope on Cry j 1, which is conformational [49,72]. The variation in allergen conformation perhaps invalidates or weakens the recognition of mAb 013 toward allergen Cry j 1. Furthermore, this might result in Cry j 1 degrading more quickly, lowering the level of allergen Cry j 1. Furthermore, the pi–sigma bonding between todomatsu oil components and allergen Cry j 1 possibly breaks or impairs the bioactivity of Cry j 1 (Figure 3 and Table A1) because of the development of pi–sigma bonds (covalent bonds) ubiquitously destroying the bioactivity of their target [70]. Consequently, the immune response of patients to allergen Cry j 1 may be lowered through reductions in the antibody reactivity, content, and bioactivity of allergen Cry j 1.

Todomatsu oil, with its characteristics of affordability, accessibility, convenience, and environmental friendliness, is an essential oil extracted from *Abies sachalinensis* waste [45]. Essential oils created from natural materials have long been applied in the culinary, perfumery, and aromatherapy industries [73–75]. Numerous essential oils, such as those from *Lavandula angustifolia* and *Cinnamomum camphora* chvar. *Borneol*, have been found to have antioxidant and antibacterial properties [75,76]. In addition, essential oils made from plants, such as *Salvia lavandulifolia* and *Melissa officinali* (France), could remove house dust mites from our living environment [74,75,77–88]. In contrast, investigations of their

inhibitory ability toward allergens are limited. It is interesting that the few plant essential oils with allergen-repressing properties that have been described were excellent at lowering allergens. Todomatsu oil displayed its capacity to decrease allergen Cry j 1. It has also been proven as an inhibitor of dominant house dust mite allergens Der f 1, Der p 1, and Der f 2 by lowering their level. In particular, its components dock to the hydrophobic amino acids of these allergens, which is considered the possible reason for their reduction. Moreover, the essential oils of citronella grass (*Cymbopogon nardus* Rendle), cinnamon (*Cinnamomum bejolghota* (Buch.-Ham.) Sweet), and clove (*Syzygium aromaticum* (L.) Merr. & L. M. Perry) diminished the allergen Der p 1 from house dust mites [89]. Accordingly, greater research should be conducted to identify additional plant essential oils that help lower allergens.

Todomatsu oil is available in various forms, such as sticks, pots, sprays, and stickers, which can be utilized both indoors and outdoors (https://www.st-c.co.jp/news/newsrelease/2021/20211215_001708.html, accessed on 10 April 2023). This versatility overcomes most limitations of existing nonpharmacological measurements and makes it a compelling alternative. The stick type, which can be applied on a mask, is good for exterior defense; the pot type is suitable for interior prevention; the spray type can be applied on the face; the sticker type can be pasted on the collar. These types are favorable for both indoor and outdoor protection, and the sticker type is appropriate for children who do not want to wear a mask. During pollen season, the combination of todomatsu oil with other measures for preventing pollen inhalation might be more reasonable and effective in avoiding both pollen grains and airborne allergens to prevent pollen allergy or relieve symptoms, such as combining the stick type with a mask and sunglasses when staying outside or the pot type with air purifiers or pollen screens when staying inside. Despite these benefits, further research, such as in vivo or epidemiological studies, is required to assess its efficacy in lowering the immune response and symptom alleviation. Additionally, its inhibition of pollen grains and other allergens should be validated in future work due to their significant contribution to triggering pollen allergy, considering the adaptability of todomatsu oil to overcome most limitations of existing avoiding methods for pollen inhalation.

Antigenicity is described as the ability to specifically bind antibodies, T-cell receptors, or major histocompatibility complex [90], i.e., the capacity to elicit an immune response [90,91]. The mAb 013 recognizes the dominant allergenic epitope on Cry j 1, with its binding to this epitope inducing a strong blockage of the IgE in the patient's serum binding to Cry j 1 [49]. Accordingly, the mAb 013 binding activity to allergen Cry j 1 partially represents the human IgE reactivity to Cry j 1. Thus, it is plausible that todomatsu oil impairs the Cry j 1 antigenicity. Further evidence for the reduced Cry j 1 antigenicity with todomatsu oil could be obtained from in vivo experiments, human serum pools, or individual sera carrying IgE. Allergen-specific immunotherapy (AIT) is currently the only clinically effective curative treatment for allergic diseases, and it provides long-lasting effects that can persist even after discontinuation [3]. However, the use of standardized JC pollen extract as a tolerogen is usually associated with severe adverse effects [3]. To address this issue, alternatives such as T-cell epitope peptides have been designed [3]. Therefore, there is speculation that Cry j 1, coupled with specific chemical molecules to lower its antigenicity, may serve as a tolerogen for AIT.

Pectate–lyase allergens from Cupressaceae pollen include Cup s 1 (*Cupressus sempervirens*) [92,93], Cup a 1 (*C. arizonica*) [92,94], Cry j 1 (*Cryptomeria japonica*) [50,92], Jun a 1 (*Juniperus ashei*) [92,95], Jun o 1 (*J. oxycedrus*), and Jun v 1 (*J. virginiana*) [92,96]. They exhibit a highly conserved three-dimensional structure [92]. In addition, they present frequent IgE-binding cross-reactivity and cross-allergenicity due to their remarkably similar conformations of IgE-binding epitopes [92]. As a result, the effectiveness of todomatsu oil in suppressing other pectate–lyase allergens from Cupressaceae pollen could be verified.

5. Conclusions

Japanese cedar (JC, *Cryptomeria japonica*) pollen allergens are responsible for causing a universal seasonal allergic illness known as JC pollinosis, a critical public health issue in Japan. Despite numerous efforts to prevent exposure to JC pollen, the study regarding the repression of JC pollen allergens is far behind. According to our data, the use of todomatsu oil (from *Abies Sachalinensis* waste) led to 85.96% and 42.12% reductions in the antibody reactivity and content, respectively, of Cry j 1, the foremost JC pollen allergen that induces JC pollinosis. Alkyl hydrophobic forces, pi–sigma bonding, and hydrogen bonding are the central driving forces underlying the docking of the todomatsu oil components with Cry j 1, according to the docking modeling. Over 50% of the amino-acid residues docked by each todomatsu oil component are hydrophobic, except for α-pinene. The oil components, particularly β-maaliene, demonstrated substantial repression of allergen Cry j 1. Therefore, todomatsu oil, with its characteristics of affordability, accessibility, convenience, and environmental friendliness, is a reasonable approach for repressing allergen Cry j 1.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. List of docking outcomes of todomatsu oil components on allergen Cry j 1 (P18632).

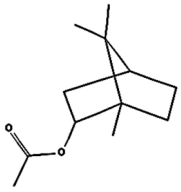
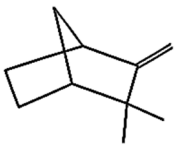
Todomatsu Oil Components	PubChem CID	Chemical Formula	Chemical Structure	Docking Energy (Kcal/mol)	Inhibition Constant (μM)	Docked Amino-Acid Residues
(a) Bornyl acetate	6448	$C_{12}H_{20}O_2$		−5.2	154.16	**AHI:** LEU262; ILE320 **HB:** THR299 **CHB:** GLU264
(b) Camphene	6616	$C_{10}H_{16}$		−5.1	182.51	**AHI:** LEU262; TYR315

Table A1. *Cont.*

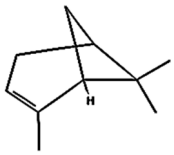
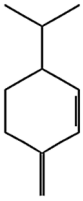
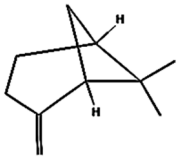
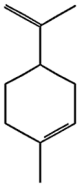
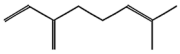
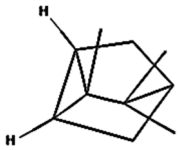
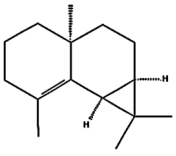
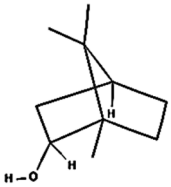
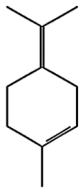
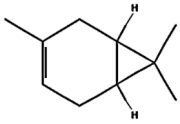
Todomatsu Oil Components	PubChem CID	Chemical Formula	Chemical Structure	Docking Energy (Kcal/mol)	Inhibition Constant (μM)	Docked Amino-Acid Residues
(c) α-Pinene	6654	$C_{10}H_{16}$		−5.3	130.22	**AHI:** TYR85; LYS111; ARG112; ILE136; PHE140
(d) β-Phellandrene	11142	$C_{10}H_{16}$		−5.3	130.22	**AHI:** LYS111; ARG112; ILE136; PHE140
(e) β-Pinene	14896	$C_{10}H_{16}$		−5.3	130.22	**AHI:** TYR85; LYS111; ILE136; PHE140
(f) Limonene	22311	$C_{10}H_{16}$		−5.4	109.99	**AHI:** LYS111; ARG112; ILE136; PHE140 **PS:** PHE140
(g) β-Myrcene	31253	$C_{10}H_{16}$		−4.9	255.80	**AHI:** LYS111; ILE136; PHE140; VAL145
(h) Tricyclene	79035	$C_{10}H_{16}$		−4.9	255.80	**AHI:** LEU262
(i) β-Maaliene	101596917	$C_{15}H_{24}$		−6.7	12.26	**AHI:** HIS239; LEU262; TYR315; ILE320
(j) Borneol	64685	$C_{10}H_{18}O$		−5.0	216.07	**AHI:** LEU262

Table A1. *Cont.*

Todomatsu Oil Components	PubChem CID	Chemical Formula	Chemical Structure	Docking Energy (Kcal/mol)	Inhibition Constant (μM)	Docked Amino-Acid Residues
(k) α-Terpinolene	11463	$C_{10}H_{16}$		−5.6	78.48	**AHI:** TYR85; LYS111; ILE136; PHE140
(l) 3-Carene	26049	$C_{10}H_{16}$		−5.5	92.91	**AHI:** TYR85; ILE136; PHE140; VAL145 **PS:** PHE140

LEU: leucine; ILE: isoleucine; THR: threonine; GLU: glutamic acid; TYR: tyrosine; LYS: lysine; ARG: arginine; PHE: phenylalanine; VAL: valine; HIS: histidine. AHI: alkyl hydrophobic force; HB: conventional hydrogen bonding; CHB: carbon hydrogen bonding; PS: pi–sigma bonding.

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