Fine mapping of \( qBlsr3d \), a quantitative trait locus conferring resistance to bacterial leaf streak in rice

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Funding information
This work was supported in part by the National Key R&D Program of China (2017YFD0100100), Natural Science Foundation of Fujian, China (2017J01438) and Regional Development Project of Fujian, China (2018N3011).

Abbreviations: \( BLS \), bacterial leaf streak; \( CDS \), coding sequence; \( CSSL \), chromosome segment substitution line; \( InDel \), insertion–deletion; \( NIL \), near isogenic line; \( PCR \), polymerase chain reaction; \( QTL \), quantitative trait loci; \( SSR \), simple sequence repeat.

Received: 5 December 2019 | Accepted: 20 March 2020
DOI: 10.1002/csc2.20155

Abstract
Bacterial leaf streak (BLS) is a destructive bacterial disease in rice (\( Oryza sativa \) L.). To date, at least 13 quantitative trait loci (QTL) conferring resistance to BLS have been identified in rice, while only one QTL, \( qBlsr5a \), has been fine mapped and cloned. The present study focuses on fine mapping of \( qBlsr3d \), a minor QTL conferring resistance to BLS. To fine map this QTL, 24 overlapping chromosome segment substitution lines (CSSLs) were developed from the \( F_2 \) population derived from H359 × H359-BLSR3D. Combining genotyping of molecular markers with resistant performance, \( qBlsr3d \) was delimited to an 81-kb interval on chromosome 3, which included 12 annotated genes. Sequence alignment indicated that one of the candidate genes, \( LOC_{Os03g03570} \), has three nucleotide substitutions in the CDS region between H359 and H359-BLSR3D. In particular, \( LOC_{Os03g03570} \) encodes a leucine-rich repeat transmembrane protein, which has been reported to be associated with disease resistance, suggesting that \( LOC_{Os03g03570} \) may be the target gene. Our research also suggests that CSSLs are suitable for mapping of minor QTL conferring disease resistance. Furthermore, our finding has potential value in breeding rice varieties with resistance to BLS in rice.

1 | INTRODUCTION

Rice is one of the most important cereals in the world. To date, there have been dozens of diseases found in rice including viral diseases (e.g., yellow mottle virus, black stripe dwarf, grassy stunt virus, tungro bacilliform virus), fungal diseases (e.g., bakanae disease, blast, sheath blight disease, false smut),
and bacterial diseases (e.g., bacterial leaf blight, bacterial leaf streak, damping off) (Zhu, Tao, & Xu, 2017). Bacterial leaf streak, which is caused by the pathogen Xanthomonas campestris pv. Oryzicola, is one of the major bacterial diseases in rice (Tang, Wu, Li, Lu, & Worland, 2000). Bacterial leaf streak was first observed in rice in China and later in tropical and subtropical rice-producing areas of Asia, and it seriously affects the yield and quality of rice (Tang et al., 2000). The most cost-effective way to control BLS is to breed resistant varieties. Some resistant cultivars, such as ‘Dular’, ‘Acc8558’, and ‘Acc8518’, have been identified in earlier studies (Xia, Lin, & Chen, 1992; Zhang, Lu, & Zhu, 1996). Based on these studies, the generally accepted conclusion is that BLS resistance in rice is controlled by QTL (Tang et al., 2000; Chen, Zheng, Huang, Zhang, & Lin, 2006; Tang, Li, & Wu, 1998), and at least 13 QTL conferring BLS resistance have been reported (Chen et al., 2006; Tang et al., 2000; Zheng, Li, & Fang, 2005). Among them, 11 QTL were identified by Tang et al. (2000). In our previous studies, qBlsr5a, a QTL reported by Tang et al. (2000) with relatively major effects that can explain 12.84–15.93% of phenotypic variation, was fine mapped in a 30-kb interval using CSSLs (Xie et al., 2014). Subsequently, we confirmed that the target gene of qBlsr5a is LOC_Os05g01710 using CRISPR-Cas9 and real-time quantitative reverse transcription polymerase chain reaction (PCR) (unpublished data, 2016). Interestingly, the possible candidate gene LOC_Os05g01710, also named xa5, is a major recessive resistance gene for bacterial leaf blight. Yuan et al. (2016) verified that this gene is involved in both resistance to bacterial blight and resistance to bacterial leaf streak in rice. Feng et al. (2016) found that the OsPGIP4 gene, which may be a potential component of the qBlsr5a locus for BLS resistance, can positively regulate the defence response of rice to BLS through activated expression of the jasmonic acid pathway.

In a natural environment, plants are constantly challenged by pathogens. For this reason, plants have developed a series of defense mechanisms against pathogens during their long evolutionary processes. According to the inheritance of plant disease resistance and the sensitivity of resistance to environmental conditions, plant disease resistance can be classified into two groups: qualitative resistance and quantitative resistance (Kou & Wang, 2010; Brun et al., 2010). Qualitative resistance, also known as vertical resistance or specific resistance, is a genetic trait that is controlled by single dominant R genes (Brun et al., 2010; Pink, 2002). Qualitative resistance genes can only resist certain physiological races of pathogens. Although qualitative resistance genes are strong, plants with such disease resistance usually exert a large selection pressure on the pathogen, thereby accelerating the development of various pathogenic factors in the pathogen population. As a result, the disease resistance in such plants may become unstable or may even be lost (Kiyosawa, 1982). In contrast, quantitative resistance, which is also called horizontal resistance, general resistance, or persistent resistance, is controlled by multiple minor genes and results in more persistent resistance to pathogens (Parlevliet & Zadoks, 1977; Liu, Niu, Deng, & Tan, 2007). To date, many of the R genes in rice have been cloned or identified, especially those for rice blast and bacterial leaf blight. In contrast, only a few QTL conferring quantitative resistance have been fine mapped, although a number of resistant QTL have been identified in rice, such as those for sheath blight (Channamallikarjuna et al., 2010; Wang, Pinson, Fjellstrom, & Tabien, 2012; Zuo et al., 2014; Zuo et al., 2013), bacterial blight (Chen et al., 2016; Zhang et al., 2015; Sujatha et al., 2011; Zhang, Yang, Jiang, Gao, & He, 2009), rice blast (Fukuoka & Okuno, 2001; Ashkani, Rafii, Rahim, & Latif, 2013; Sallaud et al., 2003), and bacterial leaf streak (Tang et al., 2000). Most of the disease-resistant genes currently used in rice production confer qualitative resistance. However, because of the evolution of pathogens, varieties containing a single qualitative resistance gene may show weakened or lost resistance after being planted for several years. Therefore, it is necessary to clone a group of resistant QTL to breed varieties with long-lasting resistance.

In our previous study, a minor QTL, qBlsr3d, was detected, and it conferred 6.39–9.78% of total variation based on a recombinant inbred population derived from Acc8558 × H359 (Tang et al., 2000). Subsequently, a near isogenic line (NIL), H359-BLSR3D, containing only the qBlsr3d region was developed by combining one crossing, three back-crossing, and two self-crossing with marker-assisted selection. qBlsr3d was initially mapped to a region of 1250 kb between simple sequence repeat (SSR) markers 3dSSR12 and 3dSSR12 on chromosome 3 (Cao, Chen, Lin, Wu, & Xie, 2014). In the present study, we fine mapped qBlsr3d using overlapping CSSLs and predicted the relevant candidate genes. The results lay a foundation for map-based cloning of the target genes.

2 | MATERIALS AND METHODS

2.1 | Plant materials and development of overlapping chromosome segment substitution lines

The parental lines used included the indica cultivars Acc8558 (highly resistant to BLS) and H359 (highly susceptible to BLS). Using Acc8558 as the donor parent and H359 as the recurrent parent, a NIL, H359R, containing three QTL (qBlsr3d, qBlsr5a, and qBlsr5b) was developed in our previous work (Chen, Wu, Jing, & Zhou, 2005). Based on this work, an NIL, H359-BLSR3d, was developed from H359R by one crossing, three back-crossings, and two self-crossings combined with marker-assisted selection (Cao et al., 2014).
It only contains the resistant allele of qBlsr3d but no other BLS-resistance QTL from Acc8558. The CSSLs were developed using a method similar to that described by Xie et al. (2014). An F2 population with 2663 individuals derived from H359 × H359-BLSR3d was used to screen for the recombinant plant. The F2 population was planted in the field, and the molecular markers 3dSSR3 and 3dSSR12 were used to screen for the recombinant seedlings, revealing a homozygous genotype (i.e., either of the two parental genotypes) at one marker and a heterozygous genotype at the other marker. The seeds of target plants were harvested separately from individual plants. In the following season, F2:3 seeds were sown in the field. One hundred twenty seeds were sown per line. The SSR markers 3dSSR3 and 3dSSR12 were used to screen for the F2:3 seedlings that showed the genotype of one parent (H359) at one marker and of the other parent (H359-BLSR3d) at the other marker. The homozygous recombinant seedlings from the same line constituted a CSSL, and these seedlings were transplanted to the field for the evaluation of their resistance to BLS.

2.2 Development of simple sequence repeat and insertion–deletion markers

The sequences of SSR markers were downloaded from Gramene (http://www.gramene.org/). To develop insertion–deletion (InDel) markers, the rice genome sequences of the indica cultivar 93-11 and the japonica cultivar Nipponbare were compared with identify InDel markers using the online program Blast2 (http://blast.ncbi.nlm.nih.gov/Blast.cgi), and then primers for amplifying the InDel sequences were designed using the software Primer 5 according to Xie et al. (2014) (Supplemental Table S1). Polymorphisms of the molecular markers between the parents were tested by PCR. The PCR products were run on 9% polyacrylamide denaturing gels, and the bands were visualized using the silver staining method (Xie et al., 2014).

2.3 Identification of phenotype and genotype

The bacterial strain used in this work was Xanthomonas oryzae pv. Oryzicola. The bacterial strain RP was provided by professor Guoying Chen at Huazhong Agricultural University. Inoculation was performed using needles at the active tillering stage of the plants. The concentration was $9 \times 10^8$ bacteria ml$^{-1}$. Inoculation was performed with plants in the field. The inoculation needle was dipped into the bacterial solution and used to prick the leaves. In order to reduce errors, leaves of the the latest five leaves on every plant were selected for inoculation. After 20 d, the lesion length of the inoculated leaves was measured, and 20 plants were measured for each CSSL to evaluate its resistant performance.

3 RESULTS

3.1 Assessment of the resistance of the parents

To identify the resistance of the two parents, lesion lengths were observed in the resistant parent H359-BLSR3D and the susceptible parent H359. The mean lesion lengths of H359-BLSR3D and H359 were 5.69 and 7.23 cm, respectively, as shown in Table 1. There was a highly significant difference

| Variety or line | Lesion length ± SD$^a$ | Evaluation of resistance$^b$ |
|----------------|-------------------------|-----------------------------|
| H359-BLSR3D    | 5.69 ± 0.83**           | R                           |
| H359           | 7.23 ± 1.04             | S                           |

$^a$At least five leaves were measured per plant, and 30 plants were measured for each of the two parents.
$^b$R, resistant to bacterial leaf streak; S susceptible to bacterial leaf streak.
**Significant at the .01 probability level.
### TABLE 2  Genotype and resistance level of selected recombinant chromosome segment substitution lines

| Line | Mean lesion length ± SD (cm) | Resistance evaluation (P < .05) | F₂ recombinant plant 3dSSR3 | 3dSSR12 | F₃ homozygous line 3dSSR3 | 3dSSR12 |
|------|----------------------------|--------------------------------|-----------------------------|---------|--------------------------|---------|
| A1   | 3.21 ± 0.27 S             | S                              | 3                           | 2       | 1                        | 2       |
| A2   | 3.31 ± 0.30 S             | S                              | 3                           | 2       | 1                        | 2       |
| A3   | 3.27 ± 0.26 S             | S                              | 3                           | 2       | 1                        | 2       |
| A10  | 3.04 ± 0.25 S             | S                              | 2                           | 3       | 2                        | 1       |
| A15  | 2.14 ± 0.16 R             | R                              | 2                           | 3       | 2                        | 1       |
| A16  | 2.44 ± 0.36 R             | R                              | 3                           | 1       | 2                        | 1       |
| A21  | 3.53 ± 0.30 S             | S                              | 1                           | 3       | 1                        | 2       |
| A23  | 3.41 ± 0.34 S             | S                              | 3                           | 2       | 1                        | 2       |
| A24  | 3.92 ± 0.33 S             | S                              | 3                           | 2       | 1                        | 2       |
| A27  | 3.20 ± 0.37 S             | S                              | 3                           | 2       | 1                        | 2       |
| A28  | 3.40 ± 0.28 S             | S                              | 3                           | 2       | 1                        | 2       |
| A29  | 3.82 ± 0.31 S             | S                              | 3                           | 2       | 1                        | 2       |
| B1   | 3.34 ± 0.15 S             | S                              | 1                           | 3       | 1                        | 2       |
| B5   | 2.37 ± 0.26 R             | R                              | 3                           | 2       | 1                        | 2       |
| B6   | 2.01 ± 0.17 R             | R                              | 1                           | 3       | 1                        | 2       |
| B7   | 2.01 ± 0.19 R             | R                              | 1                           | 3       | 1                        | 2       |
| B11  | 1.85 ± 0.19 R             | R                              | 2                           | 3       | 2                        | 1       |
| B12  | 2.26 ± 0.33 R             | R                              | 3                           | 1       | 2                        | 1       |
| B14  | 1.38 ± 0.13 R             | R                              | 1                           | 3       | 1                        | 2       |
| B15  | 1.08 ± 0.28 R             | R                              | 2                           | 3       | 2                        | 1       |
| B16  | 2.44 ± 0.36 R             | R                              | 1                           | 3       | 1                        | 2       |
| B18  | 2.54 ± 0.21 R             | R                              | 2                           | 3       | 2                        | 1       |
| B19  | 2.54 ± 0.31 R             | R                              | 2                           | 3       | 2                        | 1       |
| B20  | 2.01 ± 0.23 R             | R                              | 1                           | 3       | 1                        | 2       |
| H359 | 3.54 ± 0.32 S             | S                              | 1                           | 1       | 1                        | 1       |
| H359-BLSR3D | 1.86 ± 0.16 R       | R                              | 2                           | 2       | 2                        | 2       |

**Note.** The band-type of H359 was recorded as 1, the band-type of H359-BLSR3D was recorded as 2, and the heterozygous band-type was recorded as 3.

*R, resistant to bacterial leaf streak; S susceptible to bacterial leaf streak.*

between the two parents (P < .01). The lesion lengths of H359 were longer than those of H359-BLSR3D (Table 1).

### 3.2 Construction and resistance assessment of the chromosome segment substitution lines

Twenty-four recombinant plants were picked out from among 2,663 F₂ seedlings after genotyping with the two molecular markers: 3dSSR3 and 3dSSR12. Then, the 24 recombinant plants were harvested, and 24 homozygous CSSLs (F₃) were developed in F₂:F₃, including eight CSSLs that exhibited the genotype of H359-BLSR3D and 16 CSSLs that exhibited the genotype H359 at marker 3dSSR3 (Table 2). In addition, the 24 CSSLs could be classified into two groups based on the lesion length: 11 CSSLs were susceptible and 13 CSSLs were resistant to BLS (Table 2).

### 3.3 Fine mapping qBlSr3d

In our previous work, qBlSr3d was preliminarily mapped to a 1250-kb region on chromosome 3 (Cao et al., 2014). To further narrow down this interval, we first chose one SSR marker (3DSSR4) located at approximately the halfway point between 3DSSR3 and 3DSSR12 to analyse the 24 CSSLs. Based on the genotypes at three markers (3DSSR3, -4, and -12) and resistance phenotypes to BLS, we found that the genotypes of A15, A16, B11, B12, B15, and B18 in the interval between 3DSSR4 and 3DSSR12 contradicted their phenotypes (Supplemental Table S2; Supplemental Figure S1). Therefore, this interval could be excluded, and it could be speculated that qBlSr3d is located between markers 3DSSR3 and 3DSSR4 (spanning ~580 kb).

Next, we developed 12 SSR and nine InDel polymorphic markers between 3DSSR3 and 3DSSR4 (Supplemental
Table S1). We found that the genotypes of lines A1, A23, and A24 in the interval between ID22 and 3DSSR4 and those of B5, B6, B14, B16, B19, and B20 in the interval between RM523 and 3DSSR15 contradicted their phenotypes (Figure 1; Supplemental Table S2). Finally, we narrowed the location of $qBlsr3d$ down to the 81-kb region between RM22 and 3DSSR51.

### 3.4 | The candidate gene of $qBlsr3d$

There are 12 annotated genes in this 81-kb region according to the Rice Genome Annotation Project database (Table 3). Based on the Bioinformatics analysis of those candidate genes, the predicted product of LOC_Os3g03570 is leucine-rich repeat transmembrane protein kinase, which is reported to be associated with plant immunity. So the CDSs of LOC_Os3g03570 in the two parents were sequenced and aligned. The results indicate that there are three base substitutions between the two parents. Specifically, the coding region of LOC_Os3g03570 has single base substitutions at positions 1039, 2101, and 2113, respectively, between the two parents (Figure 2a). These three base substitutions result in changes in the protein sequence, especially at positions 701 and 705, and the amino acids changed from G to R and R to G between H359 and H359-BLSR3D (Figure 2b).

### 4 | DISCUSSION

Generally, populations used for QTL mapping can be divided into two categories: the primary population and the secondary population. The primary population mainly includes the $F_2$, backcross ($BC_1$), double haploid, and recombinant inbred lines, which were commonly used in early QTL mapping (Jiang & Zeng, 1995; Lander & Botstein, 1989; Zeng, 1993). Although the construction of the primary population is relatively simple, it may still be affected by interference from the genetic background, so it is difficult to accurately identify the effect of a QTL and its location on the chromosome. Moreover, for certain QTL with minor genetic effects, the mapping results often deviate greatly from the actual results. Secondary populations are developed by cross- and multigeneration backcross screenings, which generally exclude most of the interference from the genetic background and improve the accuracy and precision of QTL mapping (Ando et al., 2008; Shabanimofrad et al., 2017; Zhu et al., 2008; Zhao et al., 2009; Zhou et al., 2017; Zhao et al., 2007). The most
**TABLE 3** The candidate genes of qBlsr3d

| TIGR gene ID   | Gene product name                                                                 | Biological process                                                                 |
|----------------|-----------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| LOC_Os03g03480 | DUF623 domain containing protein, expressed                                        | Molecular function, cellular component, biological process                          |
| LOC_Os03g03490 | Expressed protein                                                                  | Not found                                                                           |
| LOC_Os3g03500  | Heavy metal-associated domain containing protein, expressed                         | Cellular component, binding, transport                                              |
| LOC_Os3g03510  | CAMK_KIN1/SNF1/Nim1_like.15 -CAMK includes calcium/calmodulin dependent protein kinases, expressed | Response to stress, response to abiotic stimulus, metabolic process etc.            |
| LOC_Os3g03520  | Expressed protein                                                                  | Not found                                                                           |
| LOC_Os3g03530  | Expressed protein                                                                  | Molecular function, cellular component, biological process                          |
| LOC_Os3g03540  | No apical meristem protein, putative, expressed                                    | Biosynthetic process, sequence-specific DNA binding transcription factor activity, nucleobase, nucleoside, nucleotide and nucleic acid metabolic process |
| LOC_Os3g03550  | Bzip family transcription factor, putative, expressed                              | DNA binding, biosynthetic process, cytosol, sequence-specific DNA binding transcription factor activity, etc. |
| LOC_Os3g03560  | FHA domain containing protein, putative, expressed                                  | Plastid                                                                             |
| LOC_Os3g03570  | Leucine-rich repeat transmembrane protein kinase, putative, expressed               | Signal transduction, kinase activity, nucleotide binding, protein modification process, plasma membrane |
| LOC_Os3g03590  | Transporter, monovalent cation: proton antiporter-2 family, putative, expressed     | Transport, cellular process, transporter activity                                     |
| LOC_Os3g03600  | Fasciclin-like arabinogalactan protein, putative, expressed                         | Plasma membrane, membrane                                                            |

**FIGURE 2** Sequences comparison of the gene LOC_Os03g03570 between two parents. (a) Comparison of the part coding sequences. (b) The structural domain of LOC_Os03g03570. Red arrows indicate the base substituted or amino acid changed
commonly used secondary population is the CSSL, which is a series of NILs developed by backcrossing and molecular marker-assisted selection. With the exception of the partially replaced fragments from the donor parent, the CSSL is identical to the recipient’s parent, thus eliminating the interference of the genetic background.

Bacterial leaf streak is a destructive bacterial disease in rice. Previous studies have indicated that rice resistance to BLS is controlled by QTL, especially multiple minor-effect QTL (Chen, Zheng, Huang, Zhang, & Lin, 2006; Tang et al., 2000), although a few studies have reported that some genes can increase rice resistance to BLS (Guo et al., 2012; Ju et al., 2017; Li et al., 2019). In the study by Tang et al. (2000), 11 QTL were identified, most of which were minor QTL. At present, the fine mapping of QTL is generally performed in such a way as to minimize the interference of the genetic background by constructing a secondary mapping population such as CSSLs. In our previous work, the QTL with the greatest effect, qBlsr5a, was fine mapped to a 30-kb interval using CSSLs (Xie et al., 2014). The successful mapping of qBlsr5a provides us with a reference, which demonstrates the feasibility of using CSSLs to fine map minor QTL. Based on these works, we fine mapped the QTL qBlsr3d, which confers BLS resistance in rice using CSSLs. Firstly, we developed H359-BLSR3D, a NIL that contains only the resistant allele of qBlsr3d but none of the other BLS resistance QTL from Acc8558. In phenotype assays, it was determined that the lesion length of H359-BLSR3D was significantly shorter than that of the susceptible parent, H359. Based on the genotype and resistance of H359-BLSR3D, we can conclude that H359-BLSR3D does contain the QTL qBlsr3d. Secondly, we developed 24 CSSLs using a series of molecular markers combined with field phenotypes to fine map qBlsr3d. Finally, qBlsr3d was delimited to an 81-kb interval. In this study, we used a strategy similar to the mapping of qBlsr5a, but considering that the effect value of qBlsr3d is smaller than that of qBlsr5a, we identified more homozygous individuals in F2.3 lines for the identification of resistance. The purpose of this step was to reduce the error caused by the environment. In addition, we also performed field experiments over multiple seasons to reduce the phenotypic error caused by the environment. In addition to the 24 substitution CSSLs used in this study, some CSSLs were not clearly distinguishable as resistant or susceptible to BLS in repeat experiments and were finally eliminated. Therefore, for the fine mapping of the minor resistance QTL, it is necessary to expand the mapping populations. Although a minor QTL usually exhibits a quantitative effect in primary mapping population, the phenotype can be distinguished between the CSSLs or NILs and the recurrent parent. In our study, H359-BLSR3D and 13 CSSLs showed a significant difference in resistance compared with the recurrent parent H359. The results also indicated that qBlsr3d had the potential for breeding varieties with resistance to BLS and can be used in backcrossing breeding.

The final 81-kb interval includes 12 annotated genes. One gene, LOC_Os3g03570 has three base substitutions between the two parents (Figure 2). In addition, two substitutions (2101 and 2113) occur in a conserved domain of LOC_Os3g03570 (Figure 2b). LOC_Os3g03570 is predicted to contain a leucine-rich repeat transmembrane domain that is related to plant immunity (Halter et al., 2014; Ma, Gan, & Wang, 2005; Song, Li, Song, & Zheng, 2008). It shows that LOC_Os3g03570 is more likely to be the target gene of qBlsr3d. However, this prediction is only a preliminary speculation, and further functional verification of the target gene is underway. In conclusion, based on the fact that the target gene qBlsr3d can increase resistance to BLS, the two molecular markers, RM22 and 3DSSR51, and potential SNPs developed based on polymorphisms of the candidate gene can be used in marker-assisted breeding to improve the resistance of bacterial leaf streak.

**AUTHOR CONTRIBUTIONS**

Z.C. designed the project and modified the manuscript. S.W. performed the experiments, analysed the data, and wrote the manuscript. X.X., Z.Z., H.G., D.M., and W.W. participated in the work. All authors interpreted the results and contributed to the writing.

**CONFLICTS OF INTEREST**

The authors declare no conflict of interest.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**How to cite this article:** Wang S, Xie X, Zhang Z, et al. Fine mapping of *qBlsr3d*, a quantitative trait locus conferring resistance to bacterial leaf streak in rice. *Crop Science*. 2020;1–9. [https://doi.org/10.1002/csc2.20155](https://doi.org/10.1002/csc2.20155)