Computational Prediction of Ubiquitination Proteins Using Evolutionary Profiles and Functional Domain Annotation

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Abstract: Ubiquitination, as a post-translational modification, is a crucial biological process in cell signaling, apoptosis, and localization. Identification of ubiquitination proteins is of fundamental importance for understanding the molecular mechanisms in biological systems and diseases. Although high-throughput experimental studies using mass spectrometry have identified many ubiquitination proteins and ubiquitination sites, the vast majority of ubiquitination proteins remain undiscovered, even in well-studied model organisms.

Objective: To reduce experimental costs, computational methods have been introduced to predict ubiquitination sites, but the accuracy is unsatisfactory. If it can be predicted whether a protein can be ubiquitinated or not, it will help in predicting ubiquitination sites. However, all the computational methods so far can only predict ubiquitination sites.

Methods: In this study, the first computational method for predicting ubiquitination proteins without relying on ubiquitination site prediction has been developed. The method extracts features from sequence conservation information through a grey system model, as well as functional domain annotation and subcellular localization.

Results: Together with the feature analysis and application of the relief feature selection algorithm, the results of 5-fold cross-validation on three datasets achieved a high accuracy of 90.13%, with Matthew’s correlation coefficient of 0.80.34%. The predicted results on an independent test data achieved 87.71% as accuracy and 75.43% of Matthew’s correlation coefficient, better than the prediction from the best ubiquitination site prediction tool available.

Conclusion: Our study may guide experimental design and provide useful insights for studying the mechanisms and modulation of ubiquitination pathways. The code is available at: https://github.com/Chunhuixu/UBIPredic_QWRCHX

Keywords: Ubiquitination, machine learning, random forest, protein annotation, subcellular localization, functional domain.

1. INTRODUCTION

As a well-known post-translational modification (PTM), ubiquitination is crucial in proteome dynamics and various signaling pathways in the cells [1]. Ubiquitination is an enzymatic PTM in which ubiquitin (a small regulatory protein) [2] is attached to a lysine residue of the targeting protein [3]. Ubiquitination marks proteins for degradation through the proteasome [4], alters their cellular location [5], and regulates protein interactions [5]. It is involved in signal transduction [6], apoptosis, endocytosis, gene transcription, DNA repair, and replication, intracellular trafficking, virus budding [3], cellular transformation, immune response, and inflammatory response [7].

Due to its importance and complexity, the identification of ubiquitination proteins and ubiquitination sites is highly valuable. However, experimental identification is time-consuming and expensive [8] particularly because the ubiquitination process is dynamic, rapid and reversible [9-11]. Hence, computational predictions become an important and practical alternative. A number of computational methods were developed based on the traditional machine learning method for predicting lysine ubiquitination sites, including Radijovic’s UbPred [12], Cai’s mRMR model [13], Zhao’s ensemble classifier [14], and Chen’s CKSAAP approach [15], but they can only identify ubiquitination sites with limited accuracies. It is not practical to predict ubiquitination proteins through these methods since the false-positive rates would be too high to be useful. Recently, deep learning
method tools, such as MusiteDeep-Capsule [16] have become competitive due to the advancing of computing resources, our results show good performance even when compared with it, also, it has a better performance than three machine learning-based tools, UbiProber [17], UbiSite [18] and PDM-PUB [19]. To the best of our knowledge, so far no computational method has been developed to predict whether an uncharacterized protein is able to be ubiquitinated or not. The present study was initiated in an attempt to address this problem for the first time. If it can be predicted whether an uncharacterized protein can be phosphorylated or not [20, 21]. A method has been presented for identifying human phosphorylated proteins by incorporating evolutionary information into a general pseudo amino acid composition (PseAAC) model through a grey system [21-23]. It is believed that the formulation and approach can be also used to predict ubiquitination proteins. Ubiquitination is much less frequent than phosphorylation, as ubiquitination is a much more “expensive” biological operation than phosphorylation. The smaller group of ubiquitination proteins may have even stronger common features than phosphorylation proteins so that a whole sequence-based method may work better in predicting ubiquitination proteins. Furthermore, other gene features will be used, such as Gene Ontology (GO) [24], a structured repository of concepts (GO Terms) related to gene functions for the prediction, none of which was used in predicting phosphorylation sites.

In this study, a novel computational method, was developed to predict ubiquitination proteins for a query amino acid sequence on the basis of its evolutionary information through a grey system model [25] and K Nearest Neighbour (KNN) scores calculated with the fuzzy distance by using its Functional Domain Annotation (FDA) and subcellular localization. There are two major feature sets in this study: one set includes 80 sequence grey model features extracted from the sequence evolution information and another contains the features calculated by KNN scores based on FDAs. To thoroughly evaluate the proposed model, it was trained and tested with different datasets and cross-validations methods. In addition, the distribution of the above-mentioned features in predicted ubiquitination proteins was analyzed and it provided some hypotheses for distinguishing ubiquitination proteins from non-ubiquitination ones.

2. MATERIALS AND METHODS

2.1. Benchmark Dataset

The dataset used was extracted from Uniprot at http://www.ebi.ac.uk/uniprot [26]. The version of protein data used in the current study was released on May 2017. The positive dataset containing 1906 known ubiquitination proteins was generated through the following queries in the UniProt advanced search: “annotation: (type:crosslink ubiquitin) length: [50 TO *] AND reviewed: yes.” Three hundred of 1906 proteins were separated as an independent test dataset so that the remaining 1606 positive proteins were kept in training and validation. For the negative dataset, we started from all reviewed proteins (~550,000 totals) and performed a filtering process by using CD-HIT-2D [27] with a threshold of 70%, after this step, there were 320,096 negative proteins left. To conduct balanced training, these samples were randomly taken to form three negative datasets, in which the number of samples was the same as the given positive dataset. At this time, a 300 proteins negative independent dataset was randomly selected and isolated for testing. There is no overlap between training and testing datasets. For the annotation information, we extracted the 8 types UniProt annotations of ‘Subcellular localization (SL)’ [28] and FDAs of ‘GO’ [29]’, ‘Pfam’ [30]’, ‘Smart’ [31]’, ‘PROSITE’ [32]’, ‘SUPFAM’ [33]’, ‘InterPro’ [34]’, and ‘PRINTS’ [35]’ for all the proteins in the datasets. SL was reorganized by the UniProt build-in hierarchical subcellular localization table.

2.2. Incorporate Extracted Features into the General Pseudo Amino Acid Composition

It is known that most traditional machine-learning algorithms, such as Neural Network [36], Covariant Discriminant [37], Support Vector Machine [38], K Nearest Neighbor [39], and Random Forest [40], can only handle vector but not sequence samples. To formulate a biological sequence of a variable length into a discrete model or a vector, yet still considerably keep its sequence pattern or inherent characteristics, researchers formulated the protein sequence or peptides using pseudo amino acid composition (PseAAC) [21], encoding method [41] or other approaches [42]. Here, a model following the general form of PseAAC [43] has been proposed, which formulates a protein P as (Eq. 1):

$$P = [P_1, P_2, \ldots, P_u, \ldots, P_N]^T$$

where T is a transpose operator, the subscript $\Omega$ is an integer, and its value as well as the components $P_1, P_2, \ldots$ depend on the extraction of the desired information from the amino acid sequence of P as described below.

2.3. Vectorization of Sequence Profile through a Grey System Model

From the evolutionary viewpoint, all the protein sequences have been evolved from a very limited number of ancestral species. Their evolution involves mutations of single residues, as well as insertions and deletions of residues, gene duplication, and gene fusion. With these changes accumulated for a long period of time, many similarities between the original and evolved amino acid sequences have gradually disappeared, but they may still share some common features, such as belonging to the same type of protein [44], residing in a same subcellular location [45], or having a similar biological function [46]. It is assumed that ubiquitination proteins have evolutionary relationships that are reflected in some common attributes encoded in sequence profiles, i.e. the Position Specific Scoring Matrix (PSSM), as described below. The sequence profile by a $L \times 20$ matrix as P is given as:
samples, according to local sequence similarity. For proteins subcellular localizations were also used as a feature for predicting proteins, respectively, and the distance $\text{Dist}_{ij}(p, q)$ between $p$ and $q$ is defined as follows in Eq. (4):

$$\text{Dist}_{ij}(p, q) = 1 - \frac{|\text{FDA}_{ij}(p)\cap\text{FDA}_{ij}(q)|}{|\text{FDA}_{ij}(p)\cup\text{FDA}_{ij}(q)|}$$

which represents the $j$-th feature of FDA of $p$ and $q$, respectively. $j = 1, 2, \ldots, 78$ represents ‘GO’, ‘Pfam’, ‘Smart’, ‘PROSITE’, ‘SUPFAM’, ‘InterPro’, ‘PRINTS’ or ‘subcellular localization’, respectively, and the distance $\text{Dist}_{ij}(p, q)$ between $p$ and $q$ is defined as follows in Eq. (4):

$$\text{Dist}_{ij}(p, q) = 1 - \frac{|\text{FDA}_{ij}(p)\cap\text{FDA}_{ij}(q)|}{|\text{FDA}_{ij}(p)\cup\text{FDA}_{ij}(q)|}$$

| Step 2. | A corresponding KNN feature is then extracted by calculating the KNN score, represented by the percentage of positive neighbors (ubiquitination proteins) in its $k$ nearest neighbors. |

| Step 3. | To take advantage of different properties of neighbors with various similarity cutoffs, Steps 1 and 2 were repeated for different $k$ values to obtain multiple features for the ubiquitination protein predictor. In this study, based on empirical trials, by default, $k$ was chosen to be 0.1%, 0.4%, 0.7%, ..., 14.5% and 14.8%; then the number of features is 50, i.e. 50 KNN scores were extracted as features for predicting ubiquitination proteins. For the $j$-th member of FDA, the protein $P$ can be formulated as (Eq. 5):

$$P_{\text{FDA}} = [\varphi_1(j), \varphi_2(j), \ldots, \varphi_K(j)]^T$$

where $\varphi_1(j), \varphi_2(j), \ldots, \varphi_50(j)$ are the ratios of positive neighbors to the whole samples at 0.1%, 0.4%, ..., 14.8% of the training dataset size, respectively. Hence, a query protein sequence can be formulated with seven 50-dimensional vectors, i.e., $P_{\text{FDA}} = [P_{\text{FDA}1}, P_{\text{FDA}2}, \ldots, P_{\text{FDA}50}]$ by using the FDA database and a 60- or 80-dimensional vector for each $P_{\text{FDA}k}$, i.e., $P_{\text{PSSM-Grey}}^{(60)}$ or $P_{\text{PSSM-Grey}}^{(80)}$. These digital representations are used as the input of query protein for the prediction model. |

| 2.5. Algorithm | Random Forest has been used as the main classifier of the predictor. The workflow (Fig. 1) illustrates how our classifier works. In the proposed model, the first step is to input the query amino acid sequence with its FDAs. The next step is to generate two sets of features of a given protein as described above, where the annotation features are encoded into a distance matrix based on the KNN-score extraction, and PSI-BLAST was used to generate the PSSM and then transform into the SeqEvo Descriptor. In the last step, two types of features are assembled to enter the machine learning classifier as input for training. |

| 2.6. Method Evaluation | To evaluate the prediction performance of our method, a 5-fold cross-validation test was performed following several widely-accepted measurements: (1) overall accuracy (ACC), |
of true positive and true negative sample among all the samples; (2) Mathew’s correlation coefficient or MCC; (3) sensitivity (SN), the ratio between true positive and positive samples; and (4) specificity (SP), the ratio between true negative and negative samples; (5) precision (Pre), the ratio of true positive among the sum of true positive and false positive. As mentioned in Section 2.1, there were 3 sets of negative data, and 3 sets of training data were constructed whose positive datasets were the same. Then training and 5-fold cross-validation were performed 3 times, then all these measurements were calculated from the average of 3 training sets. Furthermore, Receiver Operating Characteristic (ROC) curves were calculated and plotted based on specificities and sensitivities. The Areas under ROC curves (AUCs) were also calculated based on the trapezoidal approximation.

The GO enrichment network of the training dataset A, B, C indicates the positive datasets of *H. sapiens*, followed by *M. musculus* and *A. thaliana*, D, E, F indicate the negative datasets with the same order of species. The network was generated using Cytoscape [44], packaged in Metascape, with p-value < 0.01, minimum count 3, and enrichment factor > 1.5. An average score of 4 was used as the similarity metric when performing hierarchical clustering on the enriched terms and then sub-trees with similarity > 0.3 were considered a cluster. Each node represents an enriched cluster and colored by its similarity ID as shown in the legend. The edge indicates the number of shared proteins between two-term nodes.

### 3.2. GO Enrichment Analysis

To confirm the classification results, the gene set GO enrichment analysis was performed using Metascape [50]. Here, the analysis of 1906 positive data on three species: *Homo sapiens* (393 GO terms), *Mus musculus* (390 GO terms) and *Arabidopsis thaliana* (277 GO terms) has been performed. For 10,000 negative datasets, the numbers of GO annotations was 1059, 1034 and 639, respectively. It shows that in all three species, most ubiquitination proteins belong to a small number of biological annotation terms with small p-values, which indicates that annotations could be useful features for our machine learning approach. Figs. (2) and (3) show that for *H. sapiens* and *M. musculus*, the positive datasets are more centered in the same functional GO term groups, with many linked edges this may be due to the commonness of mammals. The negative datasets for these two species are clustered in independent groups with fewer edges. Such a pattern is less obvious in *A. thaliana*.

![Flowchart of our algorithm approach.](Flowchart.png)

### Table 1. Performance comparison of PSSM-Grey by a 5-fold cross-validation.

|        | PSSM – Grey (80) | PSSM – Grey (60) |
|--------|------------------|------------------|
|        | ACC | MCC | SN | SP | ACC | MCC | SN | SP |
| KNN    | 79.99 | 60.52 | 86.63 | 73.35 | 80.43 | 61.37 | 86.79 | 74.08 |
| SVM    | 88.68 | 77.60 | 84.75 | 92.61 | 87.62 | 75.28 | 86.00 | 89.24 |
| RF     | 86.21 | 72.44 | 87.24 | 85.19 | 86.19 | 72.41 | 87.24 | 85.15 |
| Average | 84.96 | 70.19 | 86.20 | 83.72 | 84.75 | 69.68 | 86.68 | 82.82 |

*Note: The abbreviations in the table are: Accuracy (ACC), Matthews Correlation Coefficient (MCC), Sensitivity (SN) and Specificity (SP).*
3.3. Subcellular Localization Enrichment Analysis

To study the relationships between ubiquitination proteins and subcellular localization, the enrichment analysis of subcellular localization for this dataset has been performed (Fig. 4). As shown in Fig. (4), 49.8% of the positive data were labeled with the nucleus location, and 44.3% were localized in the cytoplasm, which is significantly different from the negative data (8.1% and 26.5%, respectively). Also, it is noted that 94.4% positive proteins have more than two localization annotations and 61.1% for negative proteins, especially, for positive data which is localized in the nucleus, around half of them (460 of 950) share other localizations. Some earlier studies have shown that the ubiquitin-related enzymes are highly localization-specific [24], and hence the subcellular localization could be an informative feature to predict ubiquitination proteins.

3.4. Investigating the Performances of KNN Score of Features

It was found that 5,184 GO terms were involved in the training dataset, of which 3,012 appeared in the set of ubiquitination proteins, 3,565 appeared in non-ubiquitination proteins, and only 1,393 GO terms were shared by both positive and negative datasets. Hence, the functional properties of the two groups are significantly different, as consistently shown in Figs. (2 and 3). Following this idea, the KNN scores of ubiquitination proteins were compared with those of non-ubiquitination proteins on all the FDA features (Fig. S2). Overall, ubiquitination proteins gained obvious larger KNN scores which are greater than 0.5 (i.e., with significant information content as prediction feature; the larger, the more significant) on GO and subcellular localization, and a slightly larger score greater than 0.5 in the Smart, SUPFAM, and InterPro.

Specifically, for ubiquitination proteins, the average KNN scores of GO with different sizes of nearest neighbors were within 0.5 - 0.8, and for non-ubiquitination proteins, the average KNN scores were within 0.2 - 0.4. For subcellular localization, the average KNN scores of ubiquitination proteins were in the range of 0.5 - 0.7, while those of non-ubiquitination proteins fluctuated around 0.4. For Smart, SUPFAM, and InterPro, there was no clear gap between the ubiquitination proteins and non-ubiquitination proteins, especially with the growth of KNN cutoffs. Subsequently, the eight types of features were tested on the three datasets with KNN, RF and SVM algorithms on the training dataset, and the mean performance of these three algorithms is listed in Table 2. The best results of accuracy for RF, SVM, and KNN are 0.88 (using InterPro), 0.85 (using Pfam) and 0.85 (using Pfam), respectively. The Random Forest algorithm has the best performance on all the features of accuracy, where the accuracy is between 0.70 - 0.88. Hence, Random Forest has been selected as our classifier.

3.5. Performance of the Proposed Model

Since the combined features generated a high-dimensional vector output, the Relief method [25] can be used to rank the values of the underlying features. To
evaluate the performance of our method for different features, 5-fold cross-validation has been performed (Tables 3 and 4 for training and testing, respectively). In general, the evaluation result was the best when all features were included reaching the accuracy of 87.71% and MCC of 75.43%. The performance of the proposed models was further illustrated by the ROC analysis (Fig. 5), especially using AUC (Area Under the Curve). The AUC value is a number between 0 and 1, and the greater the AUC value, the better is the predictor. The AUC value of the proposed model is 0.8507 (Go), 0.8509 (PFAM), 0.8502 (SMART), 0.8495 (PROSITE), 0.8513 (SUPFAM), 0.8501 (INTERPRO), 0.8497 (PRINTS), 0.8476 (Subcellular localization), 0.9396 (GreyPssm) and 0.9598 (All).

3.6. Testing Data Performance and Comparison with Ubiquitination Site Prediction

A balanced independent test dataset was used to evaluate our model in comparison with ubiquitination site prediction tools. The results were based on five-fold cross-validation, with the model including all features together for our tool. The results were compared with a deep learning ubiquitination site prediction tool, MusiteDeep-Capsule [16]; a machine learning approach based tool, UbiProber [17]; an SVM
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Fig. (4). Distribution of subcellular localization of the training data. (A higher resolution / colour version of this figure is available in the electronic copy of the article).

Table 2. A comparison of eight features with different algorithms.

| Feature   | %   | ACC  | MCC  | SN   | SP   |
|-----------|-----|------|------|------|------|
|           |     | RF   | SVM  | KNN  | RF   | SVM  | KNN  | RF   | SVM  | KNN  |
| GO        | 82  | 81   | 80   | 64   | 62   | 60   | 83   | 79   | 79   | 81   | 83   | 81   |
| Pfam      | 87  | 85   | 85   | 75   | 70   | 70   | 83   | 80   | 83   | 92   | 90   | 87   |
| Smart     | 73  | 72   | 70   | 50   | 50   | 42   | 50   | 49   | 60   | 95   | 95   | 81   |
| PROSITE   | 79  | 78   | 76   | 60   | 59   | 55   | 64   | 61   | 67   | 94   | 94   | 86   |
| SUPFAM    | 75  | 73   | 74   | 52   | 48   | 50   | 60   | 56   | 60   | 90   | 90   | 88   |
| InterPro  | 88  | 83   | 83   | 77   | 67   | 67   | 86   | 79   | 81   | 91   | 88   | 86   |
| PRINTS    | 70  | 70   | 69   | 49   | 49   | 45   | 41   | 41   | 43   | 99   | 99   | 95   |
| SL*       | 80  | 78   | 77   | 59   | 57   | 54   | 76   | 78   | 76   | 83   | 79   | 77   |

Note: The abbreviations in the table are: Accuracy (ACC), Matthews Correlation Coefficient (MCC), Sensitivity (SN) and Specificity (SP). Three algorithms, Random Forest (RF), Support Vector Machine (SVM) and KNN (K-Nearest Neighbor) were applied. * indicates Subcellular Localization (SL).

Based tool, UbiSite [18]; and a Bayesian Discriminant Method based tool, BDM-PUB [19]. Since existing prediction tools are designed for site prediction, their site prediction results were transformed to ubiquitinated protein results by using the following strategy: if any site from a given protein was predicted as ‘positive’ or ‘ubiquitinated’, the whole protein was labeled as ‘positive’ as well; if multiple sites were predicted as positive, the max predicted score was picked from them for generating the Receiver Operator Curve (ROC). Their pre-trained models were used with default parameters to conduct the prediction comparison with our method. For MusiteDeep-Capsule (another in-house tool), the same test dataset was used to train the models and the same independent test dataset was used to perform the comparison. For other tools, they do not provide customized model training, therefore their pre-trained model was used to predict for the same testing dataset. The results are shown in Fig. (6), in which our tool showed a better performance than other tools; in particular, our tool has a significantly lower false-positive discovery rate and a higher true positive discovery rate than other tools.

4. DISCUSSION

In order to detect ubiquitination proteins, a method was developed based on the Random Forest algorithm using the sequence conservation information, as well as the information of ‘GO’, ‘Pfam’, ‘Smart’, ‘PROSITE’, ‘SUPFAM’, ‘InterPro’, ‘PRINTS’ and subcellular localization of the query protein. The features only incorporate the sequence conservation using a grey system model and KNN scores based on protein annotation databases. This method achieved an overall accuracy of 90.03%, MCC of 80.13%, Sn of 87.94%, Sp of 92.13% and Precision of 91.78%, which indicates that this method reflects the sequence patterns well, containing
Table 3. A comparison of eight features performance in the training data.

| Feature(1-8) | ACC    | MCC    | SN    | SP    | Precision | Recall  |
|--------------|--------|--------|-------|-------|-----------|---------|
| 1 GO         | 82.18  | 64.39  | 83.29 | 81.08 | 81.52     | 83.29   |
| 2 Pfam       | 87.35  | 75.01  | 82.88 | 91.83 | 91.03     | 82.88   |
| 3 Smart      | 72.62  | 50.45  | 50.49 | 94.75 | 90.59     | 50.49   |
| 4 PROSITE    | 78.69  | 60.24  | 63.50 | 93.89 | 91.23     | 63.50   |
| 5 SUPFAM     | 75.01  | 52.30  | 60.43 | 89.59 | 85.34     | 60.43   |
| 6 InterPro   | 88.42  | 76.91  | 86.25 | 90.59 | 90.16     | 86.25   |
| 7 PRINTS     | 70.07  | 49.06  | 41.31 | 98.83 | 97.24     | 41.31   |
| 8 SL         | 79.64  | 59.49  | 76.00 | 83.28 | 82.02     | 76.00   |
| 9 PSSM       | 86.19  | 72.40  | 87.34 | 85.04 | 85.37     | 87.34   |
| Feature(1-9) | 89.74  | 79.53  | 87.85 | 91.63 | 91.30     | 87.85   |

Note: The abbreviations in the table are: Accuracy (ACC), Matthews Correlation Coefficient (MCC), Sensitivity (SN) and Specificity (SP). “Feature(1-8)” indicates that the first 8 features were applied, and “Feature(1-9)” means that all features were applied.

the ubiquitination sites. Since our method could do the prediction without relying on sequence profiles, it can scan a batch of unknown proteins very efficiently. In addition, our method showed better performance than the existing tools for the protein level prediction of ubiquitination. The user may apply our predictor to select potential candidates before doing the site prediction or the lab work.
Fig. (5). ROC curves to show the performance of proposed models. The blue curve indicates the model with single feature and the red curve indicates the model includes all 9 features. (A higher resolution / colour version of this figure is available in the electronic copy of the article).

Fig. (6). ROC curves to show the performance comparison with other prediction tools. AUC indicates the area under the curve. (A higher resolution / colour version of this figure is available in the electronic copy of the article).
CONCLUSION
Our study may guide experimental design and provide useful insights for studying the mechanisms and modulation of ubiquitination pathways. The comparison results indicate that we have an advantage in ubiquitination prediction at the protein level. It may improve the sensitivity when conducting the ubiquitination site prediction if our method is applied first to remove the false positive samples. In addition, it may help accelerate the expensive and time-consuming process of identifying ubiquitination proteins with known annotations.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE
Not applicable.

HUMAN AND ANIMAL RIGHTS
No Animals/Humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION
Not applicable.

AVAILABILITY OF DATA AND MATERIALS
The data supporting the findings of this article is available in the GitHub at: https://github.com/Chunhuixu/UBIPredication.

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CONFLICT OF INTEREST
The authors declare no conflict of interest, financial or otherwise.

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SUPPLEMENTARY MATERIAL
Supplementary material is available on the publisher’s website along with the published article.

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