DeepShape: estimating isoform-level ribosome abundance and distribution with Ribo-seq data

Hongfei Cui¹,², Hailin Hu³, Jianyang Zeng⁴* and Ting Chen¹*

From The International Conference on Intelligent Biology and Medicine (ICIBM) 2019
Columbia, OH, USA. 09-11 June 2019

Abstract

Background: Ribosome profiling brings insight to the process of translation. A basic step in profile construction at transcript level is to map Ribo-seq data to transcripts, and then assign a huge number of multiple-mapped reads to similar isoforms. Existing methods either discard the multiple mapped-reads, or allocate them randomly, or assign them proportionally according to transcript abundance estimated from RNA-seq data.

Results: Here we present DeepShape, an RNA-seq free computational method to estimate ribosome abundance of isoforms, and simultaneously compute their ribosome profiles using a deep learning model. Our simulation results demonstrate that DeepShape can provide more accurate estimations on both ribosome abundance and profiles when compared to state-of-the-art methods. We applied DeepShape to a set of Ribo-seq data from PC3 human prostate cancer cells with and without PP242 treatment. In the four cell invasion/metastasis genes that are translationally regulated by PP242 treatment, different isoforms show very different characteristics of translational efficiency and regulation patterns. Transcript level ribosome distributions were analyzed by "Codon Residence Index (CRI)" proposed in this study to investigate the relative speed that a ribosome moves on a codon compared to its synonymous codons. We observe consistent CRI patterns in PC3 cells. We found that the translation of several codons could be regulated by PP242 treatment.

Conclusion: In summary, we demonstrate that DeepShape can serve as a powerful tool for Ribo-seq data analysis.

Keywords: Ribo-seq, Translation, Transcript-level, Multiple alignment

Background

Regulation of mRNA translation is critical to the coordinated and controlled expression of many important biological pathways, which have great impact on human health states [1, 2]. The ribosome, a complex molecular machine, plays a central role in the translation process [3]. A ribosome binding to a transcript implies the synthesis of a new peptide. Multiple factors can influence ribosome binding and also ribosome distribution at transcripts, including composition of nucleotides along its current, upstream, and downstream RNA sequences [4, 5]; instantaneous environmental stress [6, 7]; and state of the cell [8]. Therefore, distributions of ribosome binding in a transcriptome reflect underlying translation process, and thus is strongly related to phenotype state.

Ribosome sequencing (Ribo-seq) is an important approach to obtain ribosome distributions by sequencing “ribosome-protected fragments” (RPFs) [9]. Computational analysis involves mapping sequencing Ribo-seq reads into transcripts and counting number of reads at each position to obtain distributions of ribosomes along the whole transcriptome. The sites where Ribo-seq reads are piled up suggest ribosome stalling or translation slow-down, which could be caused by factors such as...
which two isoforms, 1 and 2, share the same exon, C. Besides ribosome stalling, ribosome profiles also reveal unexpected translation events on non-coding regions [14]. Overall, ribosome profiling brings insight to the process and regulation of translation.

Computational tools, or pipelines, have been developed for the analysis of Ribo-seq data, including Ribomap [15], RiboProfiling [16], RiboSeqR [17], Plastid [18] and Ribogalaxy [19]. However, most of these tools were targeted at gene-level ribosome analysis, instead of more informative transcript-level analysis. The challenge is that Ribo-seq reads (~30 bp) are too short to be mapped accurately to multiple alternatively spliced transcripts, resulting in a large proportion of multiple mapped reads [15]. This has become the main barrier against obtaining precise ribosome profiling at transcript level. A simple strategy is to discard these multiple-mapped reads. However, as Wang et al. show in their study [15], there may be less than 10% of all reads can be mapped to unique positions on transcript sequences. Discarding all multiple mapped reads would lose major information in this process. Another strategy is to allocate multiple-mapped reads randomly, as adopted by RiboSeqR [17], or the ‘star prime’ method evaluated in the work of Wang et al. [15], but this may introduce bias to transcripts of low-abundance genes, and in addition, it has been shown that this strategy does not perform well on transcript level analysis [15]. A more robust strategy, adopted by Ribomap [15], is to take advantage of RNA-seq data generated simultaneously with Ribo-seq and allocate multiple-mapped reads to transcripts according to the transcript abundance estimated from the RNA-seq data. However, this would involve two assumptions: (1) translation activity is coherent to the abundance of mRNA molecules, and (2) that ribosome profiles at regions shared by two different isoforms of a gene are exactly the same. Strictly speaking, for the first assumption, mRNA abundance is not equal to the translating mRNA abundance because some of the mRNAs may be unoccupied by any ribosomes, which is linked to different levels of initiation efficiency [9, 20], and neither is translating mRNA abundance equal to ribosome abundance on them because different translating mRNAs have different elongation velocities which lead to different ribosome densities on them [20–22]. For the second assumption, as many studies have shown, the upstream and downstream sequence compositions are important and can affect how fast ribosomes move along a transcript [4, 5].

If a frame shift exists between two isoforms because of different upstream exons, their ribosome profiles are most likely to be different. Figure 1 shows an example in which two isoforms, 1 and 2, share the same exon, C, which is connected to different upstream exons, A and B; therefore, we observe different ribosome profiles for these two isoforms, which is not considered by existing methods.

Another way to analyze ribosome profiles is to predict ribosome distributions according to transcript sequence. Liu and Song developed an effective model, RiboShape, by transforming the ribosome profiles into a low-dimensional space using a wavelet transformation and then building a sparse model to predict the transformed footprint [4]. Their predicted profiles are in a low-dimensional space from wavelet transformation, instead of at nucleotide-level resolution. This work inspired us to calculate ribosome distributions for transcripts by combination of allocating Ribo-seq reads to isoforms and predicting ribosome profile from isoform sequence.

Here, we present our model, called DeepShape, to analyze Ribo-seq data without the help of RNA-seq data. We introduced a deep learning ribosome distribution shape model to overcome heterogeneity of ribosome profiles by incorporating different upstream or downstream sequences as features to predict ribosome abundance. Multiple-mapped reads are allocated according to both pre-estimated ribosome abundances and distributions. Iteration mechanism was used for more accurate estimation of ribosome abundances and distributions for transcripts. Testing DeepShape on synthetic data shows that it can provide more accurate estimation on ribosome abundance and more precise ribosome distribution profiles than state-of-the-art methods. Using DeepShape, we analyzed a Ribo-seq dataset of the PC3 human prostate cancer cells [23] in transcript level. Isoforms of the four cell invasion/metastasis genes, which were reported to be regulated in translation by PP242 treatment in the original paper, show distinct translational efficiencies and regulation patterns. We proposed “Codon Residence Index” (CRI), which reflects the relative speed when a ribosome going through a codon compared with its synonymous codons. The CRI values between synonymous codons are significant. Several codons show at least 20% slower (higher-CRI) or faster (lower-CRI) in ribosome speed compared with the average of their synonymous codons in all the four experiments. After PP242 treatment, twelve codons represent consistently and statistically significant speed up or slowing down in both replicates. The observations validate the necessity of analyzing Ribo-seq data to produce transcript-level profiles.

Implementation
Synthetic data and real data used in this study
In this study, we generated a pair of synthetic Ribo-seq/RNA-seq sequence data to test the performance of our DeepShape method and other state-of-the-art methods.
To generate the synthetic Ribo-seq data, firstly we used published RNA-seq data from human HeLa cells (GSM546921) to calculate ground-truth transcript abundance. The transcript abundances are length-normalized, which is defined as
\[
t_i = \frac{c_{ti}}{l_{ti}} \sum_{k \in T} \frac{c_{tk}}{l_{tk}} \times 10^6,
\]
where \(c_{ti}\) is the number of RNA-seq reads on transcript \(i\), \(l_{ti}\) is the length of the \(i\)-th transcript, and \(T\) is the set of all transcripts. This index also has a name of “Transcripts Per Million (TPM)”, which is widely used in RNA-seq analysis. Human tRNA and rRNA reads were filtered out before clean reads were mapped to human HG38 transcriptome reference by STAR [24] and quantified by Salmon [25]. Secondly, we randomly generated a set of synthetic transcript efficiency (TE) values, which is the ratio of relative ribosome abundance over transcript abundance. The ribosome abundance values are normalized by coding region (CDS) length, and is defined as
\[
r_i = \frac{c_{ri}}{l_{ri}} \sum_{k \in T} \frac{c_{rk}}{l_{rk}} \times 10^6,
\]
where \(r_i\) is the CDS-length normalized abundance, \(c_{ri}\) is the number of Ribo-seq reads on transcript \(i\) (ribosome count abundance), \(l_{ri}\) is the length of the coding region of the \(i\)-th transcript, and \(T\) is the set of all transcripts. The TE values are \(r_i/l_i\). TE values are generated following a log-normal distribution, thereby enabling calculation of Ribo-Seq reads counts. Thirdly, we calculated a synthetic ribosome distribution for every transcript using a ribosome flow model [26]. Finally, we generated the synthetic Ribo-Seq reads according to their reads counts, ribosome distribution and reference transcriptome, using the “footprint_generator” program from Ribomap. To generate synthetic RNA-seq data, we applied the rlsim program [27] on the TPM obtained above. The synthetic data generation pipeline is a simulation of the real translation and sequencing process.

To show the usefulness of our DeepShape, we also reanalyzed a published PC3 human prostate cancer cell dataset [23]. The dataset consists of two replicates of PP242-treated samples and two replicates of control samples. Each of these four samples was sequenced to generate Ribo-seq and RNA-seq data. We analyzed the isoform-level translation efficiency and potential translational regulatory events using this dataset. This dataset is called “PC3 dataset” in this study.

The accuracy of the shape model in DeepShape was compared to RiboShape, using the same dataset [4]. The original Ribo-seq data come from four published datasets of \(S\). \(cerevisiae\) (yeast) treated with CHX [9, 28, 29]. Liu et al. (2016) calculated gene-level ribosome distribution using RiboShape. Genes with more than 10 continuous zero-read covered bases were discarded, resulting in 80 genes filtered out and a total of 2458 genes passing quality control. We randomly split the gene set into 3 subsets: 90% genes (2212) for training, 5% genes (124) for validation, and 5% genes (122) for testing. This dataset is called “yeast dataset” in this study. Besides, we also downloaded ribosome distribution data of three other model organisms (\(E\). \(coli\) [30], mouse [31] and human [32]) from GWIPS (https://gwips.ucc.ie/) to test the performance of our shape model. As \(E\). \(coli\) and yeast do not have alternative splicing, their datasets reflect real ribosome distribution on genes/transcripts. For alternatively spliced transcripts in mouse and human datasets, we simply joined the ribosome values in different exons to generate the ribosome distribution of transcripts.

**Designing of DeepShape-prime and DeepShape**

Our method contains two separate programs. The first one is named DeepShape-prime, which can be used for fast estimating of ribosome abundances on transcripts without considering the heterogeneity of ribosome profiles from the same exons shared by different isoforms.
DeepShape-Prime takes CDS-length-normalized ribosome abundance (see Section 2.1) as guidance of allocating multiple-map reads and updates the abundance iteratively.

As shown in Fig. 2a, initially, CDS-length-normalized ribosome abundance are set uniformly. Then, at each iteration, multiple-mapped reads are allocated proportionally to transcripts according to the current normalized ribosome abundance, and then, new ribosome abundance values are updated using the newest distributions of reads on transcripts.

The other program is called DeepShape, which differs from DeepShape-prime in that it aims to calculate more precise ribosome distributions along transcripts by allocating reads not only according to ribosome abundance, but also an instructive ribosome shape that were predicted from transcript sequences by a "shape model" that was trained from the Ribo-seq data being processed (Fig. 2b). At initiation, the normalized ribosome abundances are set uniformly, or given abundance values (say, from results of DeepShape-prime), and the instructive ribosome shapes are also set uniformly. In each iteration, DeepShape performs three tasks. At Step 1, it assigns reads according to current normalized ribosomal abundances and instructive ribosome shape values to obtain a temporary ribosome distribution. At Step 2, it trains the shape model using the temporary ribosome distributions and then applies the shape model to transcript sequences to update instructive ribosome shape values. At step 3, it updates a new set of ribosomal abundances according to the read abundance on the transcripts. This process iterates until the results are stabilized.

Design and training of shape model

In DeepShape, the shape model is a context-dependent model which characterizes ribosome moving patterns or distributions. We use a convolution neural network (CNN) model to learn the association between the context nucleotides and shape of ribosome profiles (Fig. 2c). The model was built by a Python deep learning library Keras [33].

We used the encoded codon sequence, instead of nucleotide sequence, to represent the surrounding context of the ribosome. The input to the CNN model is the

![Fig. 2 Designing of DeepShape and the shape model.](image)

A pipeline of DeepShape-prime. After a uniform initiation of ribosome abundance normalized by CDS length, in each iteration, reads are mapped proportional to normalized ribosome abundance, and then a new ribosome abundances are updated. A shape model is introduced, and in each iteration, reads are mapped proportional to ribosome abundance and an instructive ribosome shape together. Then the shape model is fitted to generate a new instructive ribosome shape which can introduce heterogeneity because of different contexts. Structure of the shape model. The model contains an embedding layer which converts the codons to 'one-hot' vectors, three convolutional layers and a full connection layer.
codon sequences of length 121 surrounding the position of ribosome (60aa upstream and 60aa downstream). The model contains a layer encoding 64 different codons into 64-dimensional 'one-hot' vectors, three convolutional layers, a full connecting layer, and an output layer. The output is the predicted instructive ribosome shape value at this site. The normalized values of ribosome distribution along transcripts were used here to train the deep learning model, which can be calculated as

\[
x_{ij} = \frac{c_{ij}}{\sum_{j \in |I|} c_{ij}} \times l_i,
\]

where \(x_{ij}\) denotes the normalized ribosome distribution of gene \(i\) at the position of its \(j\)-th codon, \(c_{ij}\) denotes the number of ribosome reads at position \(j\) on transcript \(i\), and \(l_i\) denotes the length of the coding region of the transcript. Data with extremely high or low values (outside \([\mu-3\sigma, \mu + 3\sigma]\) in log scale, \(\mu\) and \(\sigma\) are the mean and standard deviation of all values in training data) or zeros are discarded from the training dataset. Besides, the original data follows a log-normal-like distribution. We did a data re-sampling to keep the data relatively balanced. The region between \([\mu-3\sigma, \mu + 3\sigma]\) was divided into 10 bins, and in each bin, we re-sampled 50,000 data points.

We train the shape model by minimizing the following loss function:

\[
L = \frac{1}{N} \sum_{i \in |S|} \left[ \log \left( \frac{\max \left( 10^{-8}, y_{\text{predict}} \right) }{\max \left( 10^{-8}, y_{\text{ground-truth}} \right) } \right) \right]^{2},
\]

where \(N\) denotes the size of the training data. The training of the CNN model stops if the loss of the validation set in current epoch is higher than that of the last epoch.

**Comparison of the shape model and DeepShape**

We applied the shape model of DeepShape to datasets from four different organisms downloaded from GWIPS: the yeast dataset used in RiboShape [4], an E. coli dataset [30], a mouse dataset [31] and a human dataset [32]. We compared our performance with that of RiboShape.

We compared both DeepShape-prime and DeepShape with Ribomap for accuracy of the predicted ribosome distributions at the transcripts. Default parameters of Ribomap were used in this study. We also included a naive method which distributes the multiple-mapped reads uniformly to all target transcripts. As the mapping process was carried out by STAR [24], we call the naive method as "STAR-uniform" in this paper. In addition, we tested an RNA-seq quantification tool, Salmon, using the synthetic Ribo-seq data directly, in order to assess the performance of the representative RNA-seq profiling method.

**Calculation of codon residence index**

To study potential translation mechanisms, we investigated the translation speed of codons. We defined Codon Residence Index (CRI), which shows the relative speed when a ribosome going through a codon compared with its synonymous codons. This index is defined in the following:

\[
\text{CRI}_{ij} = \frac{1}{n_{ij}} \sum_{k=1}^{n_{ij}} \frac{1}{S_j} \sum_{i \in S_j} \frac{1}{n_{ij}} \sum_{k=1}^{n_{ij}} \frac{x_{ik}}{x_{ij}} - 1,
\]

where \(\text{CRI}_{ij}\) denotes the CRI value of codon \(j\) on a specific transcript \(i\), \(x_{ik}\) denotes the normalized ribosome distribution value (see Section 2.3) at the position of the \(k\)-th codon on the \(i\)-th transcript, \(n_{ij}\) denotes the number of codon \(j\) on transcript \(i\), and \(S_j\) denotes the set of codon \(j\)'s synonymous codons on that transcript (including \(j\)). If a codon has a CRI value above zero, it means that a ribosome tends to pay more time on this codon compared with its synonymous codons. On the contrary, a CRI value below zero means that a ribosome tends to move faster on the codon compared with its synonymous codons.

**Results**

**Performance of the shape model on benchmark datasets**

To assess the accuracy of ribosome shape prediction, we applied the aforementioned computational models to the yeast dataset (see Methods). Table 1 shows the average Pearson correlation coefficients (PCC) between predicted ribosome profiles and ground-truth profiles for genes with different coding length. Our CNN model in DeepShape can produce an average PCC of 0.60 in the testing dataset (Additional file 1: Figure S2). Genes from 251 aa to 500 aa in length tend to have the most accurate predictions. Liu et al. showed that prediction on lower wavelet transformation space can give higher correlations using their method. Even so, our results show a better performance (0.57–0.63 PCC) without any smoothing procedure than that of RiboShape on smoothed V7 space (0.46–0.52 PCC), and even on very smoothed V4 space (0.49–0.62 PCC) (Table 1). These results prove the advantage of our CNN model.

We also tested our shape model and RiboShape on ribosome distribution datasets of E. coli, mouse and human (Methods and Additional file 1: Supplementary Methods). In E. coli and human datasets, our shape model outperforms RiboShape (Additional file 1: Table S1). In the mouse dataset, the two methods gave similar
results. However, the predicted ribosome shape that our shape model provides is at the codon level resolution, while RiboShape can only give smoothed prediction in subspace. Moreover, our shape model has great advantage in computational time. For example, on the E. coli dataset, our shape model took less than 2 hours running a 10-fold cross validation, while RiboShape took more than 3 days.

**Performance of ribosomal abundance estimation using synthetic datasets**

We simulated a pair of synthetic Ribo-seq/RNA-seq dataset, both containing 20,000,000 reads with length 30 bps (Additional file 1: Supplementary Methods). The read length and mRNA abundance were set following a real dataset from human HeLa cells [34] (see Section 2.1), which was also used in the study of Ribomap [15]. We applied DeepShape, DeepShape-prime and Ribomap to this dataset to show their performances in predicting ribosome abundance. DeepShape and DeepShape-prime used Ribo-seq data only, while Ribomap used both Ribo-seq data and RNA-seq data. Both ribosome count abundance and CDS-length-normalized abundance (see Section 2.1) were evaluated and compared.

Figure 3 plots the PCC curves of ribosome count abundance (measured in absolute counts of ribosome-protected fragments on CDS regions) estimated by DeepShape-prime, DeepShape and Ribomap, compared with the “ground-truth” of preset ribosome abundance in the synthetic dataset. DeepShape-prime was run for 1 to 1000 iterations (blue dots) and DeepShape was run for 1 to 200 iterations. For DeepShape, two kinds of initializing ribosome abundance (see Section 2.2 in Methods) were tested: uniform (red dots), or direct results from the 200-th iteration of DeepShape-prime (purple dots).

We observe that the PCC value of DeepShape-prime improves quickly at the first 30 iterations, becoming stabilized, and then finally converging to values higher than 0.99 after 100 iterations. The convergent speed of DeepShape is a bit slower than that of DeepShape-prime at the beginning iterations because it has an additional module for shape prediction, which at the end leads to higher accuracy than DeepShape-prime (0.9960 vs. 0.9949, in the 200-th iteration). Both DeepShape and DeepShape-prime outperform Ribomap (PCC 0.9342, blue dots-dashed line) after about 10 iterations. Starting from ribosome abundance exported from DeepShape-prime can help DeepShape get better performance compared with uniform initialization (0.9982 vs 0.9960, both in the 200-th iteration). To test the accuracy of existing isoform-level RNA-seq quantification algorithms on Ribo-seq data, we applied Salmon, a widely used program, to the same dataset, and obtained a PCC value of only 0.8923, much worse than existing Ribo-seq programs. This shows that designing computational tools specifically for Ribo-seq data analysis is necessary. The performances of estimating length-normalized ribosome abundances by DeepShape-prime, DeepShape and Ribomap show similar results (Additional file 1: Figure S3). In practice, as DeepShape-prime runs much faster because it does not need to calculate the shape, and since the distinction between DeepShape and DeepShape-prime is quite small, user can choose DeepShape-prime for efficient estimation for ribosome abundance information.

Additional tests were conducted to evaluate the robustness of DeepShape-prime. We tested DeepShape-prime with 200 iterations against Ribomap on three randomly generated synthetic datasets using several measures. Table 2 shows that DeepShape-prime outperforms Ribomap in all three datasets. The performance of DeepShape-prime stabilizes at values higher than 0.99 for ribosome abundance PCC, yet the performance of Ribomap floats from around 0.87 to 0.93. The ribosome loading MSE values of DeepShape-prime are about one magnitude smaller than those of Ribomap. Moreover, from the aspect of CDS-length-normalized ribosome abundance, we observe that the performance of Ribomap relies a lot on the extent of similarity between mRNA abundance and ribosome abundance (last three lines in Table 2). The results show that DeepShape is more robust than Ribomap.

**Performance of ribosomal shape prediction**

To evaluate the performance of ribosome shape prediction, we compared the mean shape Pearson correlations

| Length (codons) | RiboShape V3 | RiboShape V4 | RiboShape V7 | Shape model (DeepShape) |
|----------------|-------------|-------------|-------------|------------------------|
| <=250          | 0.61        | 0.53        | 0.45        | 0.61                   |
| 251–500        | 0.67        | 0.62        | 0.52        | 0.63                   |
| 501–750        | 0.61        | 0.58        | 0.50        | 0.59                   |
| 751–1000       | 0.52        | 0.49        | 0.46        | 0.57                   |
| > 1001         | 0.54        | 0.51        | 0.46        | 0.58                   |

*Reported in RiboShape study [4]. The V3 and V4 spaces are highly smoothed subspaces of the original ribosome distribution profile after wavelet transformation. V3 is smoother than V4. The V7 space is also smoothed, but most similar to the original space. RiboShape performs better in a lower subspace.
of the following four models: DeepShape-prime, DeepShape, Ribomap, and a simple model, called STAR-uniform, which allocates multiple-mapped reads randomly to their mapped genes. The methods were applied and evaluated in our synthetic dataset (see Section 2.1).

Figure 4a shows the PCC curves of the ribosome shape predictions for both DeepShape-prime and DeepShape from iterations 1 to 400, as well as the PCC values by Ribomap and STAR-uniform. DeepShape has the best average shape PCC of 0.900, followed by DeepShape-prime with 0.891. The result of the STAR-uniform is 0.849, but better than that of Ribomap (0.814). This is because of the misleading information by using RNA-seq abundance, which may be very different from ribosome abundance because of variation of translation efficiency. It should be noted that DeepShape first obtained the ribosome abundance results of the 200th iteration of DeepShape-prime and then estimated the ribosome profiles, which improved the PCC value from 0.891 to 0.899 after about 10 iterations.

We observed a noticeable improvement of ribosome shape predictions for many genes. For example, from STAR-uniform to DeepShape, more than 3405 genes have a better profile with improved PCC value > 0.1. The “small” improvement from DeepShape-prime (0.891) to DeepShape (0.900) means that 421 genes have a better profile with improved PCC value > 0.1.

Figure 4b shows an example of reconstructed ribosome profiles by DeepShape, DeepShape-prime, Ribomap and STAR-uniform. The three transcripts come from gene RPLP1 (ribosomal protein, large, P1. Gene ID: ENSG00000137818). This gene contains four exons, and because the length of Exon 3 is not a multiple of three, a frame shift on Exon 4 occurs in transcript RPLP1–006 compared with transcripts RPLP1–001 and RPLP1–002; hence, the ribosome distribution at this exon varies by

![Fig. 3 PCC curves for prediction of ribosome abundance at each iteration of DeepShape and DeepShape-prime. For comparison, the PCC value of Ribomap is shown.](image)

| Evaluation                          | Method               | Dataset 1  | Dataset 2  | Dataset 3  |
|------------------------------------|----------------------|------------|------------|------------|
| Ribosome count PCC                 | DeepShape-prime      | 0.9931     | 0.9947     | 0.9932     |
|                                    | Ribomap              | 0.8952     | 0.9253     | 0.8680     |
| Ribosome count MSE                 | DeepShape-prime      | 87.546     | 70.815     | 157.187    |
|                                    | Ribomap              | 1,216,917  | 854,546    | 2,213,367  |
| CDS-Length-normalized ribosome abundance PCC | DeepShape-prime | 0.9990     | 0.9991     | 0.9981     |
|                                    | Ribomap              | 0.9158     | 0.9381     | 0.9616     |
| Ground-truth mRNA vs ribosome abundance PCC (both normalized by length) |                     | 0.6775     | 0.7162     | 0.8252     |

PCC: Pearson correlation coefficient between prediction and ground-truth.
MSE: Mean square error between prediction and ground-truth.
transcripts. After introducing the shape model, we observe that the ribosome distribution of RPLP1–001 and RPLP1–002 improves significantly compared to the “ground-truth” when generating the synthetic data (Fig. 4b, light green bars) from DeepShape-prime (Fig. 4b, red line) by the increase of PCC value of RPLP1–001 from 0.828 to 0.966 and the increase of PCC value of RPLP1–002 from 0.795 to 0.961. The improvement solely results from better ribosome distribution prediction. DeepShape-prime allocates multiple-mapped reads proportionally according to ribosome abundance; therefore, in its results, the ribosome distribution shapes on exon 4 are similar among the three transcripts, as the proportions are dominated by the one with highest abundance (the third transcript here). In contrast, the DeepShape model catches the frame shift in computing the ribosome profile.

Figure 4c shows the distributions of Pearson correlations between predicted gene profiles and the ground-truth. Results show that DeepShape has significantly higher PCC values compared to those of both Ribomap and STAR-uniform. Ribosome profiles of more than 68.9% (13,823 out of 20,073) of isoforms can be reconstructed with Pearson correlation > 0.9 by DeepShape-prime.

**Translation process revealed by DeepShape**

*Discovery of ribosomal stalling using synthetic datasets*

We demonstrate that DeepShape can be used to discover ribosomal stalling events, defined as the positions at which ribosome profiles are higher than $\mu + 2\sigma$, where $\mu$ and $\sigma$ are the mean and standard deviation of the normalized ribosome distribution. We first annotated stalling events in the ground-truth ribosome profiles of the
multiple-mapped reads by RNA-seq transcript abundance is not suitable.

In the original paper of the PC3 dataset, four cell invasion/metastasis genes were reported to be significantly regulated in translation by PP242 treatment: VIM, YB1, MTA1, and CD44. This gene level observation is confirmed in our study on transcript level (Table 3). Meanwhile, we found that isoforms of the same gene may have very different TE or TE regulation patterns (Table 3). For example, four isoforms were found in VIM: VIM-001, VIM-002, VIM-003 and VIM-201 (Additional file 1: Figure S5). Among them, VIM-001 and VIM-201 have the same protein coding region, and we therefore combined them into one. VIM-002 and VIM-003 are alternatively spliced transcripts and annotated as nonsense-mediated decay (NMD), which is regarded as erroneous splicing and will be decayed under normal circumstances. In the two replicates of experiments, VIM-001 (VIM-201) shows similar TEs and TE regulation patterns after PP242 treatment, and the two NMD transcripts show very different TEs but similar TE regulation patterns (Table 3). Both NMD transcripts of VIM genes show lower TEs in replicate 2, and the effect of down-regulation by PP242 is more obvious than that of replicate 1 (Additional file 1: Table S2). The MTA1 gene also have similar phenomenon. The isoform switching also occurs in other cell types (Additional file 1: Table S3). Because of the lack of isoform-level translation studies in the past, in-depth study on the difference of TE among isoforms from the same gene is absent from the literature. In view of this, the analysis here may provide a new paradigm for the study of translation.

Translation process analyzed by combining RNC-seq with Ribo-seq
Codon usage is one of the main factors related to the speed of ribosome movement along transcripts [37]. The speed of translating a codon is supposed to be related to the current cellular concentration of its corresponding tRNA [38, 39]. As outer environmental stress may affect tRNA concentration [40], it may also affect codon-specific ribosome movement speed. Here we designed an index, named “Codon Residence Index” (CRI, see Section 2.5), which stands for the relative time usage when a ribosome passing by a codon comparing with its synonymous codons. Using CRIs calculated from the ribosome distribution reported by DeepShape, we analyzed the association of PP242 treatment and ribosomes’ movement in codon-resolution.

To avoid inaccurate ribosome distribution estimation per codon on isoforms with low abundance, we only considered isoforms with both length-normalized ribosome and mRNA abundance higher than 10. Figure 5e
shows the 64 codons’ average CRI values among all filtered transcripts. The four PC3 experiments show very similar CRI patterns (Fig. 5e). It’s common that a codon shows obvious speed up (lower CRI) or pause (higher CRI) compared with its synonymous codons. For example, the ribosome’s resident times on GCC, GGC, CGC and AGC are at least 20% shorter than on their synonymous codons (CRI ≤ 0.2), in all the four experiments. On the contrary, the ribosomes averagely stay at least 20% longer on GCA, TTA and CGA than on their synonymous codons (CRI ≥ 0.2).

CRI patterns of the same replicate are more similar compared with that of the same condition (treat/control), which imply that each cell has their relatively characterized CRI pattern. However, after PP242 treatment, the trend of CRI shifts of the two replicates shows some
interesting phenomenon: five codons show significant ribosome speed up in both replicates, and on the contrary, seven codons show significant ribosome slowing down. For all the four codons with CRI lower than −0.2 (GCC, GGC, CGC and AGC), the ribosome resident time on three of them (GCC, GGC and AGC) were significantly increased (Fig. 5, red stars) in both replicates. The other codon, GCC, also has increased average CRI values in both replicates after PP242 treatment. This implies potential regulatory mechanism of outer environment stress on codon-specific translation.

Discussion
In this paper, we developed a computational method, DeepShape, which not only accurately estimates ribosome abundance at isoform level, but also provides precise ribosome distribution profiles along transcripts. DeepShape uses Ribo-seq reads only, making full use of multiple-mapped reads.

Many excellent RNA-seq quantification tools for isoform-level abundance estimation are available, such as cufflinks [41], RSEM [42], Salmon [25] and eXpress [43]. However, Ribo-seq data have characteristics that are very different from those of RNA-seq data. For example, the location of Ribo-seq reads is actually in the upstream of real ribosome binding site, and most Ribo-seq reads are targeted to the CDS region. The main source of bias of Ribo-seq reads comes from the movement of ribosomes, not RNA-seq sequence bias. For all these reasons, existing RNA-seq tools cannot be directly applied to Ribo-seq data analysis.

The movement of ribosome is highly non-uniform, but at exon-level, the overall non-uniformity of coverage can be smoothed. Compared with allocating multiple mapped reads according to mRNA abundance, DeepShape is a more accurate approach to making use of all mapped reads. Results on synthetic data proved its superiority over the state-of-the-art methods, and the analytic results for a published human prostate cancer cell dataset show many novel observations of cancer-related, isoform-level translational regulation events, which may point to new biological discoveries.

DeepShape was developed for model organisms that have complete and accurate transcript annotations. For non-model organisms with incomplete or erroneous transcript annotations, the accuracy of our model will be affected. We hope to investigate this topic in the future.

Some parameters in DeepShape can be adjusted by users. For example, the input length of the shape model used in this study is 121 codons. However, users can manually set this number to other lengths. According to the sequence depth of Ribo-seq dataset, users can also adjust the bin number and data number in the re-sampling step when training the shape model. The number of iterations in running DeepShape-prime and DeepShape can also be defined by users. For faster computation, user can set a smaller number.

In this study, we also proposed a codon-resolution index, Codon Residence Index (CRI) to study the relative ribosome moving speed on each codon compared with its synonymous codons. Using the ribosome distribution given by DeepShape, we found some interesting CRI pattern in human PC3 cells, including potential regulation pattern that related with PP242 treatment. However, the mechanisms of CRI patterns need to be further investigated. For example, we’ve also calculated the CRI patterns of a human A549 cell and a human HBE cell [20, 22]. Results show that the two cell-lines has very similar CRI pattern, yet distinct from the patterns of the PC3 cells in this study (Additional file 1: Figure S6). This implies that the CRI pattern may not be cell-line-specific, but rather related to different experimental protocols. The suggestion also reminds us to think about the necessity of considering and eliminating the experiment-specific bias when reading the underlying translation regulation mechanisms from ribosome distribution data.
Conclusion
We propose \textit{DeepShape} to analyze Ribo-seq data, and estimate ribosome abundance and distribution at isoform level. \textit{DeepShape} makes full use of multiple-mapped reads. Compared with another state-of-art method, Ribomap, \textit{DeepShape} needs only Ribo-seq data, without any RNA-seq data. \textit{DeepShape} can help researchers to understand translation mechanisms.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12859-019-3244-0.

Additional file 1: Supplementary Methods. Figure S1. Pipeline for generating synthetic data. Figure S2. Distribution of Pearson correlation coefficients (PCC) between predicted ribosome distributions by the shape model in DeepShape and the ground-truth in training, validation and testing datasets. Figure S3. PCC curves for prediction of length-normalized ribosome abundance at each iteration of DeepShape and DeepShape-prime. Figure S4. Application of DeepShape to annotating ribosome-stalling events. Figure S5. Four isoforms of the VIM gene. Figure S6. Codon Residence Index (CRI) of different codons in A549 and HBE cell. The data are from the work of Lian et al. (Lian, et al., 2016). Figure S7. Diagrammatic explanation of finding P-sites. Table S1. Performance of shape model on genes with different lengths in E. coli. Table S2. TE and TE fold changes after PP242 treatment in the four cell invasion/metastasis genes. Table S3. mRNA abundance, ribosome abundance and TEs in the four cell invasion/metastasis genes of A549 and H1299 cells.

Abbreviations
CDS: Coding sequence; CNN: Convolution neural network; CRI: Codon residence index; mTOR: Mammalian target of rapamycin; PCC: Pearson correlation coefficients; Ribo-seq: Ribosome sequencing; RPFs: Ribosome-protected fragments; TE: Transcript efficiency; TPM: Transcripts per million

Acknowledgements
We would like to thank Professor Xuegong Zhang, Dr. Sai Zhang, Dr. Zhixing Feng, Dr. Xi Wang, Mr. Xinyun Ma and Mr. Ruochi Zhang for useful discussions.

About this supplement
This article has been published as part of \textit{BMC Bioinformatics} Volume 20 Supplement 24, 2019: \textit{The International Conference on Intelligent Biology and Medicine (ICIBM) 2019}. The full contents of the supplement are available online at https://bmcbioinformatics.biomedcentral.com/articles/supplements/volume-20-supplement-24

Authors’ contributions
TC and JZ initiated and designed the study. HC did all the experiments. HC, HH, JJ and TC analyzed the results. HC and TC wrote the manuscript. All authors have read and approved the final manuscript.

Funding
This work and publication costs are funded by the National Natural Science Foundation of China (Nos: 61872218, 61673241, and 61721003), Beijing National Research Center for Information Science and Technology (BNRist), China’s Youth 1000-Talent Program, and Beijing Advanced Innovation Center for Structural Biology.

Availability of data and materials
Source codes: https://github.com/cuihf06/DeepShape. (This is an open source software for all academic or non-academic users. Operating system: Linux; Programming language: Python.) Ribosome shape data of yeast, E. coli, mouse and human: https://gwips.ucc.ie/ Human HeLa RNA-seq data for synthetic data generation (Gene Expression Omnibus): GSM46921.

Human real PC3 data (Sequence Read Archive): SRR403882, SRR403883, SRR403886-SRR403889, SRR403892, SRR403893.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not Applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1Institute for Artificial Intelligence and Department of Computer Science and Technology, Tsinghua University, Beijing, China. 2DonLinks School of Economics and Management, University of Science and Technology Beijing, Beijing, China. 3School of Medicine, Tsinghua University, Beijing, China. 4Institute for Interdisciplinary Information Sciences, Tsinghua University, Beijing, China.

Published: 20 December 2019

References
1. Ingolia NT. Ribosome profiling: new views of translation, from single codons to genome scale. Nat Rev Genet. 2014;15(3):205–13.
2. Brar GA, Weissman JS. Ribosome profiling reveals the what, when, where and how of protein synthesis. Nat Rev Mol Cell Biol. 2015;16(11):651–64.
3. Ramakrishnan V. Ribosome structure and the mechanism of translation. Cell. 2002;108(4):557–72.
4. Liu TY, Song YS. Prediction of ribosome footprint profile shapes from transcript sequences. Bioinformatics. 2016;32(12):i183–91.
5. Zhang S, Hu H, Zhou J, He X, Jiang T, Zeng J. Analysis of ribosome stalling and translation elongation dynamics by deep learning. Cell Syst. 2017;5(3):212–20.e216.
6. Geraschenko MV, Lobanov AV, Gladyshev VN. Genome-wide ribosome profiling reveals complex translational regulation in response to oxidative stress. Proc Natl Acad Sci U S A. 2012;109(43):17394–9.
7. Liu B, Han Y, Qian SB. Cotranslational response to proteotoxic stress by elongation pausing of ribosomes. Mol Cell. 2013;49(3):453–63.
8. Sachs AB. Cell cycle-dependent translation initiation: IRES elements prevail. Cell. 2000;101(3):243–5.
9. Ingolia NT, Ghaemmaghami S, Newman JR, Weissman JS. Genome-wide analysis in vivo of translation with nucleotide resolution using ribosome profiling. Science. 2009;324(5924):218–23.
10. Chaney JL, Clark PL. Roles for synonymous codon usage in protein biogenesis. Annu Rev Biophys. 2015;44:143–66.
11. Artieri CG, Fraser HB. Accounting for biases in riboprofiling data indicates a major role for proline in stalling translation. Genome Res. 2014;24(12):2011–21.
12. Wilson DN, Arenz S, Beckmann R. Translation regulation via nascent polypeptide-mediated ribosome stalling. Curr Opin Struct Biol. 2016;37:123–33.
13. Wang Z, Sachs MS. Ribosomal stalling is responsible for arginine-specific translational attenuation in Neurospora crassa. Mol Cell Biol. 1997;17(9):4904–13.
14. Ruiz-Dera J, Messegue X, Subirana JA, Alba MM. Long non-coding RNAs as a source of new peptides. Elife. 2014;3:e03523.
15. Wang H, McM anus J, Kingsford C. Isoform-level ribosome occupancy estimation guided by transcript abundance with Ribomap. Bioinformatics. 2016;32(12):1880–2.
16. Popa A, Lebrigand K, Paquet A, Nottet N, Robbe-Sermesant K, Waldmann R, Barbry P. Riboprofiling: a Biocomputer package for standard Ribo-seq pipeline processing. F1000Res. 2016;5:1309.
17. Chung BY, Hardcastle TJ, Jones JD, Irigoien N, Firth AE, Baulcombe DC, Brierley I. The use of duplex-specific nuclease in ribosome profiling and a user-friendly software package for Ribo-seq data analysis. Rna. 2015;21(10):1731–45.
18. Dunn JG, Weissman JS. Plastid: nucleotide-resolution analysis of next-generation sequencing and genomics data. BMC Genomics. 2016;17(1):958.
19. Michel AM, Mullan JP, Velayudhan V, O’Connor PB, Donohue CA, Baranov PV. RiboGalaxy: a browser based platform for the alignment, analysis and visualization of ribosome profiling data. RNA Biol. 2016;13(3):316–9.

20. Wang T, Cui Y, Jin J, Guo J, Wang G, Yin X, He QY, Zhang G. Translating mRNAs strongly correlate to proteins in a multivariate manner and their translation ratios are phenotype specific. Nucleic Acids Res. 2013;41(9):e743–54.

21. Liu W, Xiang L, Zheng T, Jin J, Zhang G. TranslatomeDB: a comprehensive database and cloud-based analysis platform for translatome sequencing data. Nucleic Acids Res. 2018;46(D1):D206–12.

22. Lian X, Guo J, Gu W, Cui Y, Zhong J, Jin J, He QY, Wang T, Zhang G. Genome-wide and experimental resolution of relative translation elongation speed at individual gene level in human cells. PLoS Genet. 2016;12(2):e1005901.

23. Hsieh AC, Liu Y, Edlind MP, Ingolia NT, Janes MR, Sher A, Shi EY, Stumpf CR, Christensen C, Bonham MJ, et al. The translational landscape of mTOR signalling steers cancer initiation and metastasis. Nature. 2012;485(7396):55–61.

24. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR. STAR: ultrafast universal RNA-seq aligner. Bioinformatics. 2013;29(1):15–21.

25. Patro R, Duggal G, Love MI, Irizarry RA, Kingsford C. Salmon provides fast and bias-aware quantification of transcript expression. Nat Methods. 2017;14(4):417–9.

26. Reuveni S, Meilijson I, Kupiec M, Ruppin E, Tuller T. Genome-scale analysis of translation elongation with a ribosome flow model. PLoS Comput Biol. 2011;7(9):e1002127.

27. Sipos B, Slodkowicz G, Massingham T, Goldman N. Realistic simulations reveal extensive sample-specificity of RNA-seq biases. Quant Biol. 2013. https://arxiv.org/abs/1308.3172.

28. Albert FW, Muzzey D, Weissman JS, Kruglyak L. Genetic influences on translation in yeast. PLoS Genet. 2014;10(10):e1004692.

29. McManus CJ, May GE, Spealman P, Shteyman A. Ribosome profiling reveals post-transcriptional buffering of divergent gene expression in yeast. Genome Res. 2014;24(3):422–30.

30. Mohammad F, Woolstenhulme CJ, Green R, Buskirk AR. Clarifying the translational pausing landscape in bacteria by ribosome profiling. Cell Rep. 2016;14(4):686–94.

31. Castelo-Szekely V, Arpat AB, Janich P, Gatfield D. Translational contributions to tissue specificity in rhythmic and constitutive gene expression. Genome Biol. 2017;18(1):116.

32. Iwasaki S, Iwasaki W, Takahashi M, Sakamoto A, Watanabe C, Shichino Y, Floor SN, Fujiwara K, Mito M, Dodo K, et al. The translation inhibitor Rocaglamide targets a bimolecular cavity between eIF4A and Polypurine RNA. Mol Cell. 2019;73(4):738–48 e739.

33. Chollet F, Others. Keras. 2015. Available from: https://keras.io. https://keras.io/getting-started/faq/#how-should-i-cite-keras.

34. Guo H, Ingolia NT, Weissman JS, Bartel DP. Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature. 2010;466(7308):835–40.

35. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, Arora VK, Kaushik P, Cerami E, Reva B, et al. Integrative genomic profiling of human prostate cancer. Cancer Cell. 2010;18(1):11–22.

36. Guertin DA, Sabatini DM. Defining the role of mTOR in cancer. Cancer Cell. 2007;12(1):9–22.

37. Novoa EM, Ribas de Pouplana L. Speeding with control: codon usage, tRNAs, and ribosomes. Trends Genet. 2012;28(1):57–81.

38. Anderson WF. The effect of tRNA concentration on the rate of protein synthesis. Proc Natl Acad Sci U S A. 1969;62(2):566–73.

39. Tuller T, Carni A, Vestjans K, Navon S, Dorfan Y, Zalonske J, Pan T, Dahan O, Fuerman L, Pilpel Y. An evolutionarily conserved mechanism for controlling the efficiency of protein translation. Cell. 2010;141(2):344–54.

40. Dittmar KA, Goodenbour JM, Pan T. Tissue-specific differences in human transfer RNA expression. PLoS Genet. 2006;2(12):e221.

41. Trapnell C, Williams BA, Pertea G, Mortazavi A, Kivan C, van Baren MJ, Salberg CL, Wold BJ, Pachter L. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol. 2010;28(5):511–5.

42. Li B, Ruotti V, Stewart RM, Thomson JG, Dewey CN. RNA-Seq gene expression estimation with read mapping uncertainty. Bioinformatics. 2010;26(4):493–500.

43. Roberts A, Pachter L. Streaming fragment assignment for real-time analysis of sequencing experiments. Nat Methods. 2013;10(1):71–3.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.