SITE2Vec: A reference frame invariant algorithm for vector embedding of protein-ligand binding sites

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ABSTRACT

Protein-ligand interaction is one of the fundamental molecular interactions of living systems. Proteins are the building blocks of functions in life at the molecular level. Ligands are small molecules that interact with proteins at specific regions on the surface of proteins called binding sites. Understanding the physicochemical properties of ligand-binding sites is very important in the field of drug discovery as well as understanding biological systems. Protein-ligand binding site plays an essential role in the interaction between protein and ligand that is necessary for any living system to survive. Comparing similarities between binding sites has been one of the main focus areas since the last decade in the fields of structural bioinformatics and drug discovery. In this regard, several computational methods have been developed to compare binding sites so far. Binding site comparison requires fast and efficient method as the amount of three-dimensional protein structural information is increasing rapidly nowadays. We report in this study, development of Site2Vec, a novel machine learning-based method for reference frame invariant ligand-independent vector embedding of the 3D structure of a protein-ligand binding site. Each binding site is represented in a $d$-dimensional vector form. The 3D structures of binding sites are mapped to vector form such that similar binding sites hash into proximal localities, and dissimilar sites fall across diverse regions. A sensitivity analysis of rotation and perturbation and validation study is performed to understand the behavior of the method. Benchmarking exercises have been carried out against state of the art binding site comparison methods on state of the art datasets. The exercises validate our proposed method and demonstrate that the proposed method is rotationally invariant and can handle natural perturbations expected in the biological system. In this proposed method, prior alignment of protein-ligand binding sites is not required for vector generation and comparison of binding sites. The method is suitable for high performance computation due to its speed.

1 Introduction

Protein performs its functions by interacting with ligands and other small molecules. Protein-ligand interaction plays an important role in any biological system. Comparing similarities between binding sites has been the main focus area since the last decade to predict protein functionality in the field of structural bioinformatics and drug discovery [1]. The recent increase in structural information of molecules in Protein Data Bank [2] is influencing the use of Machine Learning in comparing protein-ligand binding sites [3].

For comparison of chemical and structural similarities of binding sites and classification of binding sites, several methods [4, 5, 6, 7] have been developed. There are two types of site comparison methods, alignment-dependent methods and alignment-free methods [8]. Alignment dependent methods provide detailed information on the atomic mapping of protein structures. They are suitable for pairwise structural similarity checking, but a proximity search
of similar sites is not possible with the alignment dependent binding site comparison methods. Alignment-dependent approaches take much time compared to alignment-free methods to calculate the similarity of binding sites. There are three major steps in alignment-dependent approaches [4]. First, the structural information of the binding site is mapped to meaningful 3D coordinates, and points are labeled based on the geometric and chemical properties. Next, two 3D templates of binding sites are superimposed using clique detection [9] and geometric hashing [10]. Finally, a score is evaluated using score function based on similar features. Probris [4], constructs a graph from a protein structure, residues are represented as vertices, and each vertex is labeled with a physicochemical property. Comparison of two proteins is performed by constructing product graph, and binding sites are examined by maximum clique algorithm, and finally, a score is calculated using a scoring function that provides the similarity measure of binding sites pair.

Alignment-free methods are efficient concerning time compared to alignment-dependent methods. They serve as high throughput screening methods for comparison of pockets in protein structure. Still, they fail to give details comparison of pocket structures, and these alignment-free methods suffer from the expectation of false-positive while comparison. These methods generate signature out of protein-ligand binding sites, and those signatures are used to compare sites. PocketMatch algorithm [11] represents the binding site as lists containing distances among $C_\alpha$, $C_\beta$ and centroid of atoms of side chain and categorized the distances based on the five groups of amino acids. Other methods like spherical harmonic based [5] have been used to generate descriptors of binding sites. Fuzcav [6], alignment-free describes a binding site as a 4833 integer vector that counts the number of pharmacophoric triplet from $C_\alpha$ atom position of residues of binding sites. Another alignment-free algorithm [7] maps a binding site into 11-dimensional vector.

As the increase in structural information of molecules in Protein Data Bank [2] recently, computational Drug discovery has gained much traction in the recent decade [12] owing to the application of advanced approaches in machine learning [13]. In the field of bioinformatics, computational drug discovery, deep learning techniques are being used [14] to solve many problems like compound property and activity prediction [15], protein-ligand interaction predictions [16]. DeepDrug3D [17], a CNN based binding site classification method, represents a binding site in a 3D grid Convolution neural network processes to generate vector encoding of classes. The network uses regression formulation to learn weights from data. As a prepossessing step, to account binding site rotation, a normalized pose is determined based on three principle axes. However, the process has drawbacks. It requires an enormous amount of data as well as class information priory. And the time of execution is high.

The distribution of its atoms characterizes the shape of a binding site. All pair distances of atoms, combination of chemical properties result in an efficient shape descriptor [11]. However, Pocket match vectors are of variable in length across different sites as the descriptor is dependent on the number of atoms. Here, we propose a sliding window based method for length normalization.

In this work, we have developed site2vec, a reference frame invariant, ligand-free machine learning-based method of vector encoding of protein ligand-binding sites. The proposed method deduces a protein-ligand binding site descriptor, generated by PocketMatch Algorithm and then normalizes the descriptor to a fixed-length such that similar binding sites have been represented by similar fixed-length vectors and non-similar sites have different vector representations. Using these vector fingerprints generated by our proposed method, several binding sites are compared together at a time. The method presented here performs better than an alignment-free, vector signature-based method [7] on pairwise comparison of binding sites as well as our method is able to classify binding sites as good as DeepDrug3D [17], Deep learning-based protein-ligand binding site comparison method. Validation study on rotation and perturbation of binding sites is performed. And it is found that our method is robust in nature with respect to rotation and perturbation. It is also observed that our proposed method is much faster than state of the art, CNN based binding site comparison method, DeepDrug3D [17].

2 Methods and materials

2.1 Site2Vec: Sliding window based encoding

The proposed method, Site2Vec, takes a binding site and encode into d-dimensional vector representation. The steps are as follows (Refer Figure. [1]).

1. Representation of atoms in a binding site

Let $e$ be the binding site in dataset $\Gamma$ and $A(e)$ be the atoms of $e$.

\[ A(e) = \{(x_i, y_i, z_i, \phi_i, \psi_i) | i \in [1..N]\} \]

where,

\[ \phi_i \in \{\alpha, \beta, \gamma\} \]

\[ \alpha : C_\alpha \]
\[ \beta : C_{\beta} \]
\[ \gamma : \text{Centroid of the side chain.} \]
\[ \psi_i \in [1.k] \]
where, \( k = 5 \) [11].
\( N \) is number of atoms.

2. Calculation of pairwise distances among the atoms of binding site
\[ D(e) = \{(\text{dist}(i,j), \text{type}(i,j))|\forall i, j \in [1..N]\} \]
where,
\[ \text{dist}(i,j) : \sqrt{\sum_{p \in \{x,y,z\}}(A(e)[i].p - A(e)[j].p)^2} \]
\[ \text{type}(i,j) : A(e)[i].\phi_A(e)[j].\phi_A(e)[i].\psi_A(e)[j].\psi \]
such that,
\[ \alpha_\beta \equiv \beta_\alpha \]
\[ \alpha_\gamma \equiv \gamma_\alpha \]
\[ \beta_\gamma \equiv \gamma_\beta \]
\[ \psi_a_\psi_b \equiv \psi_b_\psi_a (\forall \psi_a \neq \psi_b) \]

3. Grouping of distances
Let \(|m|\) be the number of groups.
\[ \forall m \in \{x[1]|x \in D\} \]
\[ L_m(e) = [x[0]|x \in D, x[1] = m] \wedge (\forall a < b \rightarrow L_m[a] \leq L_m[b] \]

4. Sliding a sliding window of size \( \xi \) over the group of distances (calculated in the previous step)
\[ S^\xi_m(e) = [L_m(e)[i : i + \xi]|1 \leq i \leq |L_m(e)| - \xi + 1] \]

5. Creating bag of Features.
\[ S_m = \{ S^\xi_m(e)|e \in \Gamma(Dataset)\} \]

6. Quantization
\[ O_m = K\text{means}(S_m, k) \]

7. Mapping of each list (groups) to a histogram
\[ \forall e \in \Gamma \]
\[ C_m(e) = [O_m, \text{nearest}(s)|s \in S^\xi_m(e)] \]
\[ H_m(e) = \text{hist}(C_m(e)) \]
where,
\[ \text{nearest}(\cdot) \text{ returns nearest cluster number [13].} \]
\[ \text{hist}(\cdot) \text{ function generates histogram of cluster numbers.} \]

8. Feeding histogram to an auto encoder [19], results \( d \)-dimensional vector encoding
\[ S^\xi_{m,d}(e) = \Psi(H_m(e), \theta^*) \]
where,
\[ \Psi: \text{is an auto encoder} \]
\[ \theta^* : \arg \min_{\theta} \sum_{e \in \Gamma} ||\Psi(H_m(e)) - H_m(e)||^2 \]
\[ S^\xi_{m,d}(e) : d\text{-dimensional vector representation of binding site } e \text{ using auto encoder } \Psi. \]

2.1.1 Training process
In our proposed method, Site2Vec, a sliding window (refer step[4]) passes over the list representation of binding sites (refer step[3]) that generates equal length slices of distances of \( C_\alpha, C_\beta \) and \( \gamma \) (refer step[4]) and the slices of distances are mapped to \( k \) clusters such that each slice maps exactly one cluster (refer step[7]). The default setting of this practice includes the size of sliding window \( \xi = 10 \) (refer step[4]) and the number of clusters \( k = 10 \) (refer step[6]). The dimension of vector descriptor of binding site \( d = 200 \) (refer step[8]) where a protein-ligand binding site is represented as \( m \) number of lists where \( m = 120 \) [11] (refer step[4]).
A 1D auto-encoder network is trained that results a d dimensional vector encoding of binding sites (refer step 8). The Auto-encoder consists of four hidden layers, so total of six layers, including input and output layers followed by ReLU activation function. The configuration of autoencoder is 1200 × 1000 × 800 × 600 × 400 × 200. The network is trained on batch size of 16 with learning rate of 0.0001. The setup mentioned above is used throughout the study.

2.2 Simple mean-variance based Vector representation

In the previous section 2.1, we have mentioned that each binding is represented as a \( d \)-dimensional vector. In an intermediate (step 3) in section 2.1, a site is described as \( m \) groups of distances between atoms. A very simple approach we make to generate a vector out of groups, mentioned in step 3 is to calculate mean and variance of each \( m \) group. The pseudo-code of this mean-variance based method is described in Algorithm 1.

**Algorithm 1 Mean-Variance Method**

**Input:** \( e \) be a binding site.

**Output:** \( e_v \): Vector representation of binding site.

1. \( e_\mu = [\text{MEAN}(L_i(e))]_{i=1}^{m} \) (\( L_i \), same as step 3 in section 2.1)
2. \( e_\sigma = [\text{VARIANCE}(L_i(e))]_{i=1}^{m} \) (\( L_i \), same as step 3 in section 2.1)
3. \( e_v = e_\mu \cdot e_\sigma \)

2.3 Uniform-Site2Vec: Uniform distribution of centroids based binding site encoding method

In section 2.1 Site2Vec method is discussed and in step 6, \textit{kmeans} clustering algorithm [20] is used for grouping of \( S_m \) (refer step 5 in 2.1) in \( k \) clusters. Another simple and time-effective approach is to select uniformly \( k \) pseudo centers in \( \xi \) space that represent \( k \) clusters. The rest of the steps as well as training process (2.1.1) are same as 2.1. This version of method is named as uniform-Site2Vec.

2.4 Discretized distance and histogram based binding site representation method

In this approach, distances between \( C_\alpha, C_\beta \) and \( \gamma \) (Step 2) are discretised into seven intervals [8]. Table 1 indicates details of the division of distance range.

| Interval | Distance range (in Å) |
|----------|-----------------------|
| 1        | 0 - 7.6               |
| 2        | 7.6 - 10.1            |
| 3        | 10.1 - 12.3           |
| 4        | 12.3 - 14.3           |
| 5        | 14.3 - 16.8           |
| 6        | 16.8 - 20.0           |
| 7        | > 20.0                |

And the interval numbers associated with distances are mapped to appropriate groups based on the two atom type and its residue type (refer step 2 and 3). And finally, for each group (\( L_m(e) \)), histogram of the interval number is generated. The details of this method are described in Algorithm 2.

2.5 Datasets

To study the performance of algorithms, high quality benchmarking datasets are essential [25]. In our study, three data sets have been used to evaluate the performance measure of Site2Vec. These datasets are explained in the below.

2.5.1 TOUGH-C1 data set

TOUGH-C1 dataset is compiled by [26]. TOUGH-C1 data set consists of nucleotide- and heme-binding pockets. There are a lot of molecules that interact with nucleotide and heme- ligands in Protein Data Bank [2]. The TOUCH-C1 data set consists of one thousand five hundred and fifty-three nucleotide pockets and five ninety-six heme- binding sites.
Figure 1: Steps of Site2Vec for vector encoding of binding sites. Example of binding sites are in PDB id 1EFA and 1KFA. The illusion is created by MOL* and RCSB PDB.
**Algorithm 2** Discretized distance and histogram based method

**Input:** $e$ be a binding site.

**Output:** $v^B_e$: Vector representation of binding site.

1. $B = [\beta_1, \beta_2, \ldots, \beta_7]$ such that, $\forall i \leq 6, sup(\beta_i) < inf(\beta_i)$
2. $X_i(e) = [\beta \mid \exists \beta \in B: L_i(e)[j] \in (\beta)[L_i(e)]]$
3. $X_e = [X_i(e)]_{i=1}^m$
4. $v^B_e = [hist(X_i(e))]_{i=1}^m$

(*hist(*) returns histogram of bin numbers.)

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Table 2: Performance measure of different encoding methods on classification of nucleotide- and heme-binding sites.

| Method                  | Accuracy | AUC  | Precision | Recall | Time   |
|-------------------------|----------|------|-----------|--------|--------|
| Site2Vec method         | 0.90     | 0.92 | 0.88      | 0.71   | 323 ms |
| Mean variance method    | 0.81     | 0.83 | 0.67      | 0.61   | 92 ms  |
| Uniform-Site2Vec        | 0.88     | 0.90 | 0.84      | 0.71   | 296 ms |
| Discretized distance method | 0.86    | 0.90 | 0.87      | 0.62   | 87 ms  |

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2.5.2 PLIC: Protein-ligand interaction clusters

PLIC, Protein-ligand interaction clusters is a relational database that categorizes ligand-binding sites based on different attributes [27]. The above-mentioned database contains 67550 unique protein-ligand binding sites divided into 10854 groups based on pocket shape, types of residues, atomic contacts, and binding energy, as mentioned in [27]. Among these binding site groups, five groups that have more than 300 binding sites have been extracted for evaluating this method as any machine learning model requires a sufficient amount of data to learn each class properly. The first group has five hundred and thirty-seven binding sites. Second, third, fourth, and fifth have five hundred and thirteen, four hundred and seventy-three, three hundred and eighty-four, three forty-two binding sites, respectively.

2.5.3 ApocS3 Dataset

ApocS3 data set is composed of subject and control data set, and the Subject and Control set consists of sites from 2090 and 21660 unique protein chains from the PDB, respectively [28]. There are 38,066 pairs of binding sites in subject (positive pairs), and 38,066 pairs of binding sites are also in control (negative pairs) data set. The subject data set contains protein-ligand binding site pairs that interact with the same or similar type of ligands. And the control data set consists of binding site pairs interacting with dissimilar types of ligands.

3 Result

3.1 Comparison of variants of proposed methodology on TOUGH-C1 Dataset

To compare different methods of binding site encoding, TOUGH-C1 dataset is used. TOUGH-C1 consists of nucleotide- and heme-binding pockets [26]. There are 1553 nucleotide- and 596 heme-binding sites.

Here, heme-binding sites are treated as positive class data, and nucleotide sites are considered as negative class. The whole dataset is dividend into 70% train data and 30% test data. From the binding site structures, methods, described in section 2 are used to generate vector signatures and classifiers are used to classify the sites using the vector representations of ligand-binding sites. Table 2 reports the performance of binding site vector encoding methods, and the performance is measured using accuracy and area under ROC. The fifth column, Time, is the average time taken to encode a binding site (excluding the training time).

It is observed that Site2Vec is performing better than other methods. Site2Vec classify binding site with an accuracy of 0.90 compares to the second-best method, uniform-Site2Vec achieves an accuracy value of 0.88. The discretized distance-based encoding method has got 86% accuracy, and 81% accuracy is achieved by Mean-variance based method. From this exercise, we can clearly say that Site2Vec is performing better for the classification of binding sites on TOUGH-C1 Dataset.
3.2 Evaluation of quality of Vector representation on ApocS3 DataSet

ApocS3 data set consists of 38,066 pairs of binding sides in subject (positive pairs) as well as 38066 pairs of sites in control (negative pairs) set. The subject data set contains protein-ligand binding site pairs that interact with the same or similar type of ligands, and the control data set consists of binding site pairs interacting with different ligands [28].

Using Site2Vec, vector fingerprints of all 23750 binding sides are generated. To find similarity between two sites, the whole dataset (Subject and Control dataset) is divided into two halves. One is used as train data, and the other half is treated as test data such that both train and test set have an equivalent amount of subject and control data. The pair of binding sites in train data are fed to a classifier to train the similarity between two ligand-binding sites, and test data is used to predict the similarity score. And the performance is measured by Receiver Operating Characteristic (ROC) curve and its corresponding area under the curve. We compare our Site2Vec to state of the art alignment-free protein-ligand binding site comparison method [7] that deduces an 11 dimensional vector from a binding site and compare two sites by calculating the inner product of two vectors.

The result is displayed in figure 2. Our proposed method achieves area under the curve of 0.983, and [7] method has AUC of 0.912. It is clearly visible that our Site2Vec method performs better than Nakamura method of pairwise binding site comparison. Our method is capable of multiple comparisons of binding sites at a single point of time as the proposed method convert binding sites to vectors in $d-$ dimensional space and compares vector representations in that multidimensional space.

Table 3: Performance measure of Site2Vec and nakamura method on comparing binding sites.

| Method            | Accuracy | AUC  | Precision | Recall |
|-------------------|----------|------|-----------|--------|
| Site2vec          | 0.93     | 0.98 | 0.94      | 0.93   |
| Nakamura method   | 0.84     | 0.91 | 0.80      | 0.89   |

3.3 Benchmarking against DeepDrug3D

A benchmarking excise of Site2Vec method is performed against DeepDrug3D [17] on TOUGH-C1 and PLIC dataset.

DeepDrug3D method binding site is represented as a voxel representation with a 3D grid. For each grid point, interaction energy is calculated for 14 atom types. Heme binding sites are treated as positive class data, and nucleotide sites are considered as negative class. The whole dataset of TOUGH-C1 is divided into 70% train data and 30% test data. Site2Vec is used to generate vector fingerprint from the binding site structure, and a classifier is used to classify protein-ligand binding sites using their vector form. The performance is measured using the ROC curve.

The resultant ROC is shown in figure 3. Site2Vec has got AUC of 0.92, and DeepDrug3D achieves AUC of 0.89.

In terms of AUC, our method is performed better, and in the lower region (1% < FPR < 35 %), our approach performs better than DeepDrug3D. Table 4 gives more insight into the performance of Site2Vec and DeepDrug3D on TOUGH-C1 dataset. We can conclude that Site2Vec performs well on TOUGH-C1 dataset.

Table 4: Performance measure of Site2Vec and DeepDrug3D on the classification of nucleotide- and heme-binding sites.

| Method          | Accuracy | AUC  | Precision | Recall |
|-----------------|----------|------|-----------|--------|
| Site2Vec method | 0.90     | 0.92 | 0.88      | 0.71   |
| DeepDrug3D      | 0.84     | 0.89 | 0.85      | 0.89   |

Figure 2: ROC plot and its corresponding AUC values measuring the performance of Apos and our method on Apos dataset. The x-axis is false positive rate, and the y-axis is true positive rate.
From the PLIC database, five groups that have more than three hundred binding sites have been extracted for this study. These groups are divided based on pocket shape, nature of residues. There are total 2249 protein-ligand binding sites, and among these sites, 1507 sites are used to train the model that generate proper clusters and to train the autoencoder. And rest 742 pockets are treated as test data (test data size is 30%). The vector fingerprint, generated by our method, is used in the evaluation process of our method, Site2Vec. The vector representations of binding sites in training data are fed to a simple classifier. The use of a simple classifier is to measure the purity of vector signature. The performance of this method is measured by ROC curve and its corresponding area under the curve. Random Forest classifier classifies the vector representation of binding sites with an accuracy of 0.98.

A bench-marking exercise of the proposed method is performed with the Deepdrug3D [17] algorithm, state of the art deep learning-based binding site comparison method, to evaluate the performance of our method, Site2Vec. Figure 3 indicates the ROC curve for PLIC dataset. We can see that both Deepdrug3D (AUC 0.991) and our proposed method, Site2Vec (AUC 0.993), perform very well.

Apart from random forest classifier, other classifiers are also considered while performing the evaluation process. K-nearest neighbor classifier, Decision tree [29] are used to assess the performance of vector representation of binding sites generated by the proposed method on the classification of protein-ligand binding sites. Table 5 shows the accuracy, area under the curve of ROC plot of the proposed method, and Deepdrug3D on PLIC dataset. It is validated that our method of vector fingerprint generation of the binding site is as good as state of the art binding site comparison method on PLIC data set (Five classes extracted from PLIC database).

Table 5: Performance analysis of Site2Vec and Deepdrug3D to classify binding sites on PLIC data set. Benchmarking exercise on PLIC dataset.

| Method           | Accuracy | AUC   |
|------------------|----------|-------|
| K-NN on Site2Vec | 0.97     | 0.990 |
| Decision tree on Site2Vec | 0.95 | 0.976 |
| Random Forest on Site2Vec | **0.98** | **0.992** |
| DeepDrug3D       | 0.96     | 0.991 |

The previous exercises are performed to evaluate the computational accuracy against state of the art method on the different data sets. The third benchmarking exercise is performed to measure the Preprocessing and prediction time of the proposed method and deepdrug3D algorithm. Twenty binding sites are chosen randomly from PLIC data set and prediction time is calculated using both our method and deepdrug3D (Table 6). The average to taken by our method to generate vector encoding of a protein-ligand binding site is 0.36 second. To classify binding sites, Deepdrug3D takes average 1228.24 seconds on PLIC dataset. It is visible that our proposed method is very fast compared to Deepdrug3D algorithm.
Table 6: Benchmarking exercise on processing and predicting time performance.

| Binding Site | Site2Vec (in Seconds) | DeepDrug3D (in Seconds) |
|--------------|-----------------------|-------------------------|
| 2hb1_HEM     | 0.38                  | 220.31                  |
| 2aoc_2NC     | 0.38                  | 585.11                  |
| 1d1y_HEM     | 0.36                  | 744.8                   |
| 1d1y_HEM     | 0.37                  | 921.12                  |
| 3nlh_H4B     | 0.34                  | 316.42                  |
| 1d1w_HEM     | 0.36                  | 1216.83                 |
| 3ufr_H4B     | 0.34                  | 424.4                   |
| 1dm6_HEM     | 0.36                  | 1635.52                 |
| 1ajv_NMB     | 0.37                  | 1646.55                 |
| 3nlh_H4B     | 0.35                  | 377.18                  |
| 3nlg_H4B     | 0.39                  | 414.54                  |
| 2dn2_HEM     | 0.36                  | 1161.48                 |
| 2hbs_HEM     | 0.36                  | 1270.25                 |
| 1d1x_HEM     | 0.37                  | 1956.92                 |
| 1d0c_HEM     | 0.37                  | 1979.79                 |
| 2nnp_ROC     | 0.37                  | 2083.72                 |
| 1z1h_HBB     | 0.37                  | 1900.64                 |
| 2zlu_HEM     | 0.38                  | 1678.98                 |
| 2zlw_HEM     | 0.35                  | 1813.41                 |
| 2pwc_G3G     | 0.38                  | 2216.99                 |

Protein-ligand binding site vector fingerprint obtained by our proposed method can be visualized using the t-Distributed Stochastic Neighbor Embedding (t-SNE) [30]. The t-SNE is a tool to visualize high dimensional data by converting it into lower dimensional data. In Figure 5, two hundred pockets from each group are colored based on the group it belongs to. The binding site signatures are separable and forming five clusters as there are five different groups of binding sites. It is analyzed that our method, Site2Vec, generating vector out of binding site, embed binding sites into vector signature quiet efficiently as in t-SNE visualization similar binding site are mapped together, and different groups of binding sites are separable. It can be concluded that for similar binding sites, our method generates quite identical vector representations. For dissimilar sites, vector embedding of sites are different and using our method, and one can compare several binding sites together at a single point of time.

4 Discussion

4.1 Metrics for comparison of binding site pair

Protein-ligand binding site comparison methods use different techniques to compare sites. RAPMAD method [31] uses Jensen-Shannon divergence technique to carry out the similarity. PocketMatch algorithm uses an incremental based counting method to get a similarity score. FuzCav compares sites by calculating the number of common non-null counts in two fingerprints of ligand-binding sites [8].

In Site2Vec method, these below techniques are used to compare two binding sites.
\[
\text{cosineSimilarity} = \frac{\mathbf{A} \cdot \mathbf{B}}{\| \mathbf{A} \| \| \mathbf{B} \|} = \frac{\sum_{i=0}^{d-1} A_i B_i}{\sqrt{\sum_{i=0}^{d-1} A_i^2} \sqrt{\sum_{i=0}^{d-1} B_i^2}}
\]

where,

\( \mathbf{A} \) and \( \mathbf{B} \): Vector representation of protein-ligand binding sites.

\( d \): The dimension of vector embedding of the binding site.

The cosine similarity ranges between -1 and 1. The value -1 implies that two vectors are opposite, and one indicates that the two vectors are same and similarity value 0 indication orthogonality. The intermediate values indicate intermediate similarity.

Euclidean distance between the pair of vector signatures of binding sites is used to calculate the similarity between two binding sites.

\[
e(\mathbf{A}, \mathbf{B}) = \sqrt{\sum_{i=0}^{d-1} (A_i - B_i)^2}
\]

where,

\( \mathbf{A} \) and \( \mathbf{B} \): Vector signatures of two binding sites.

\( e(\mathbf{A}, \mathbf{B}) \): Euclidean distance between \( \mathbf{A} \) and \( \mathbf{B} \).

\( d \): Dimension of vector representation of the binding site.

These two comparison techniques are used in the sensitivity analysis of this method.

### 4.2 Sensitivity Studies

#### 4.2.1 Sensitivity analysis in respect of the rotation of binding site

This exercise is performed to analyze the behavior of the proposed method with respect to the rotation of binding sites. We chose heme-1a2sA binding site and rotate the ligand-binding site by random amount of rotation 250 times with respect to the XY- and XZ- plane.

Euclidean distance between the vector of the rotated site and vector of the original site is calculated (Figure 6). It is observed that for each rotation, the euclidean distance between the original binding site and rotated binding site is zero as it is a straight line with intensity zero. The proposed method generates similar vector encoding irrespective of any rotation of binding site. So from this result, we can conclude that our method is robust with respect to the rotation of binding sites.

#### 4.2.2 Sensitivity in respect of perturbation of coordinate of an atom of the binding site

The idea behind the experiment is to understand the behavior of our proposed method, Site2Vec, with respect to the perturbations of the binding site points(atoms).

To execute the exercise, 1a00-HEM site is chosen from PLC dataset, and we perturbed the site point between 1 Å and 4 Å randomly about 400 times. And the vector representation of each perturbed site is generated by our proposed method after that cosine similarity values between each perturbed site and the original site are calculated. Figure 7 indicate the histogram of cosine similarity values.
similarity values obtained after perturbation of 1a00-HEM binding site of amount between 1 Å and 4 Å. The x-axis denotes the cosine similarity value, and the y-axis indicates the number of perturbed binding sites having the same cosine similarity value.

In Figure 7, it is visible that approximately One hundred and forty perturbed sites have similar vector representation as to the original site. Most of the cosine similarity values lie between 0.9995 and 1, and it means that the vector representations of perturbed binding sites are very similar to the vector representation of the original site. Mean cosine similarity value is 0.9998. This experiment indicates that the proposed algorithm generates similar vector encoding on small perturbation of binding sites. That means it is resistant to small perturbation of binding sites, and our method can retain the rest of the structure of the binding site.

4.2.3 Sensitivity analysis in respect of perturbation of residue types of the binding site

In the previous exercise, single point(atom) perturbation of binding site is performed, and it is found that the proposed method is robust with respect to small perturbation of atoms. It is also important to analyze the sensitivity of the proposed algorithm while perturbing residue type (changing the nature of residue) without disturbing the coordinate of atoms of the binding site. The exercise is started with selecting a binding site 1a3n-HEM binding, and one residue is chosen randomly and changed with another residue randomly. One thousand random perturbations are carried out, and the cosine similarity values between perturbed sites and the original site are calculated. The distribution of similarity value is shown in Figure 8. The x-axis defines the cosine similarity values, and the y-axis is the number of perturbed sites.

Figure 8 shows that there is a change in the vector representation of the original site and perturbed binding sites. Most cosine values lie between 0.95 to 0.99 as the vector descriptor retains the structural representation of the binding site.

4.3 Site2Vec as a online Web Service

Site2Vec web service is an online tool, provides an interactive interface for Site2Vec, the proposed method in this paper. It provides the user to encode 3D structure of the binding site in protein to 200- dimensional vector for comparison and classification.

User is allowed to upload PDB file or provide PDB id that is downloaded from Protein data bank, and vector representations of binding sites, present in the uploaded protein structure, are computed and available for the
user to download. Multiple file upload is also present in the tool. There are more than sixty-five thousand pre-compiled vectors of binding sites available for download. There is a new feature that a user can use, to visualize the relationship of several binding sites as a dendrogram representation by uploading more than one site (vector representations generated by Site2Vec method). This web tool also provides the user to find similar binding sites of a site provided as input.

This web service is developed using python. Python Flask is used as a web framework in the backend, and the front end uses bootstrap.js libraries to facilitate the interactive interface.

5 Conclusion

In this work, we have presented Site2Vec, a machine learning-based, reference frame invariant, ligand-independent method for vector embedding of protein-ligand binding sites. This method encodes a protein-ligand binding site as d-dimensional vector. The vector representation of the site captures both the chemical and structural properties of binding sites. The quality of vector representations of protein-ligand binding sites is measured. Classification on encoded vectors of binding sites is performed to evaluate the performance of this method against a deep learning-based method on PLIC dataset and TOUGH-C1 dataset. Apart from that, Site2Vec method outperforms a state of the art alignment-free method that deduces a binding site into the 11-dimensional vector on ApocS3 dataset. A benchmarking exercise is performed that shows that our method is very fast compared to a state of the art method. An exercise is done to understand the behavior of the proposed method with respect to the rotation of binding sites. And it is validated that the method is rotation invariant and independent of any reference frame. A study on perturbation is carried out concerning binding site atoms by changing the actual coordinate or position of atoms in the site as well as perturbation of residue type of a point in the binding site without changing the actual structure of the binding site. The experiments result that our method, Site2Vec, is efficient in handling natural perturbation, expected in biological systems. We have also developed an online web tool that provides an interactive interface for vectorization and comparison of binding sites.

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