

MODELING CELL PROLIFERATION ACTIVITY OF HUMAN INTERLEUKIN-3 UPON SINGLE RESIDUE REPLACEMENTS

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Abstract: The signaling molecule human interleukin-3 (IL-3) is responsible for promoting the growth of a wide range of hematopoietic cell lineages in the bone marrow. In this study, we apply an *in silico* mutagenesis technique to investigate the effects of single amino acid substitutions in the IL-3 protein on cell proliferation activity. The computational mutagenesis, which utilizes the IL-3 protein structure as well as a knowledge-based, four-body statistical potential, empirically quantifies environmental perturbations at the mutated residue position in IL-3 and at all neighboring positions in the folded structure. In particular, mutated position perturbation scores alone are capable of characterizing IL-3 residues grouped by physicochemical, functional, or structural properties. Additionally, these scores elucidate an IL-3 structure–function relationship based on a collection of 630 single residue replacements for which activity changes were experimentally measured. A random forest classifier trained on this dataset of experimental mutants, whose respective feature vectors include environmental changes at the mutated position and at six nearest neighbors in the IL-3 structure, achieves 80% accuracy and outperforms related state-of-the-art methods.

1 INTRODUCTION

Human interleukin-3 (IL-3) is a short-chain, bundled four-helical cytokine that is produced primarily by activated T-cells and acts in the bone marrow to promote the growth of most precursor blood cell lineages (Bagley et al., 1996, Feng et al., 1996, Klein et al., 1997, Olins et al., 1995). It is a relatively small signaling protein consisting of 133 amino acid residues (Figure 1A) that most closely resembles granulocyte-macrophage colony stimulating factor (GM-CSF) and IL-5, both of which also possess four-helical bundles and belong to the same family of short-chain cytokines (Bagley et al., 1996, Feng et al., 1996). Unlike the other members of this family, a short fifth α -helix is also apparent in the IL-3 structure (Feng et al., 1996, Klein et al., 1997). Cell proliferation activity is initiated via the binding of IL-3 by a heterodimeric IL-3R α /R β transmembrane receptor on target cells (Bagley et al., 1996, Klein et al., 1997). IL-3 specifically binds the R α receptor subunit with low-affinity, and it otherwise displays no affinity for the R β chain; high-affinity IL-3 binding requires both

receptor subunits and the formation of an IL-3-IL-3R α /R β ternary complex (Bagley et al., 1996, Klein et al., 1997). Signal transduction is subsequently mediated by the R β receptor, whereby tyrosine phosphorylation of the R β cytoplasmic domain by JAK2 kinase is followed by induction of the STAT5 transcriptional pathway (Bagley et al., 1996, Feng et al., 1996, Klein et al., 1997).

A solution structure has been determined for a multiply substituted and truncated variant of human IL-3 consisting of residue positions 14 – 125 (Feng et al., 1996). The NMR coordinates, deposited into the Protein Data Bank (PDB) under accession code 1jli (Berman et al., 2000), provide a minimized average structure obtained from a family of 25 convergent structures with an average backbone root-mean-square deviation of 0.88 ± 0.15 angstroms (Feng et al., 1996). Although a total of 14 residue changes were introduced into the truncated protein in order to make it sufficiently soluble and stable for NMR studies, a cell proliferation assay revealed the variant to be fully active (Feng et al., 1996). Additionally, the results of saturation (Olins et al., 1995) and site-directed (Bagley et al., 1996)

mutagenesis experiments on IL-3 have been reported in the literature, whereby cell proliferation assays were used for measuring the activity associated with a total of 630 single residue substitutions in the native protein. The synthesized IL-3 mutants were subsequently categorized based on their degree of activity relative to that of the wild type protein.

In this study, we implement a computational mutagenesis procedure for representing mutants of human IL-3 due to single amino acid replacements. The method utilizes a coarse-grained depiction of protein structure as a collection of constituent amino acid residue $C\alpha$ coordinates in 3-dimensional (3D) space. For each structure, the points serve as vertices for a 3D tetrahedral tiling known as a Delaunay tessellation (de Berg et al., 2008); hence, every tetrahedron identifies a quadruplet of residues at the vertices. Initially, a large and diverse set of protein structures is tessellated, from which an amino acid four-body potential is subsequently developed based on statistical analysis of the residue quadruplets collectively generated by the tetrahedra. Next, we describe the *in silico* mutagenesis technique and how its application to a protein such as IL-3 requires both tessellation of its 3D structure and use of the four-body statistical potential. For each single residue replacement in IL-3, the method quantifies ensuing environmental perturbations at the mutated position and at structurally nearby positions that form a local neighborhood as identified by the tessellated protein structure. As will be shown in this manuscript, these perturbation scores elucidate IL-3 structure-function relationships and are valuable for developing a predictive model of mutant IL-3 activity based on implementation of a random forest classifier.

2 MATERIALS AND METHODS

2.1 Experimental Data

Theoretically, there are a total of $19 \times 112 = 2128$ possible single residue substitutions that can be introduced into positions 14 – 125 of the available human IL-3 structure. The principal dataset for this study contains 630 of these IL-3 mutants, representing amino acid replacements distributed throughout the primary sequence of the protein at all but 12 positions. Biological activity of these experimentally synthesized IL-3 mutants was determined via cell proliferation assays that measured the incorporation of [^3H] thymidine into either AML193.1.3 (Olins et al., 1995) or TF-1 (Bagley et al., 1996) erythroleukemic cell lines.

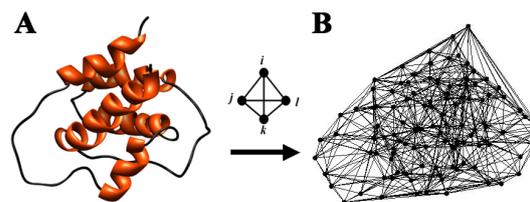


Figure 1: (A) Ribbon diagram of human interleukin-3 (IL-3) based on PDB accession code 1jli (Pettersen et al., 2004). (B) Delaunay tessellation of IL-3 using $C\alpha$ vertices generates a convex hull of tetrahedral simplices.

Mutant IL-3 activity was reported as a percentage of the wild type (wt) protein, summarized by the following categorical distribution: 27 “increased activity” mutants (> 100% wt), 373 “full activity” mutants (20 – 100% wt), 75 “moderate activity” mutants (5 – 19% wt), and 155 “low activity” mutants (< 5% wt). As a two-class system of IL-3 mutants, we consider the following subsets: 400 that are “unaffected” (“increased” and “full” combined) and 230 that are “affected” (“moderate” and “low” combined) by their respective residue substitutions.

2.2 Delaunay Tessellation and the Four-Body Statistical Potential

The Delaunay tessellation of a set of points $P = \{x_1, x_2, x_3, \dots, x_N\}$ in 3D Euclidean space yields a convex hull of space-filling, non-overlapping, irregular tetrahedra whose combined vertices consist of precisely all elements of P (Figure 1B). Two adjacent tetrahedral simplices in a tessellation may share a triangular face (three out of four points in common), a linear edge (two points in common), or a single vertex. Provided that no three points of P are collinear, no four points are coplanar or on the same circle, and no five points are on the same sphere, there exists a unique Delaunay tessellation of P (de Berg et al., 2008). The technique is applied to a protein structure by initially abstracting to points the constituent amino acids, which for this study are selected to be the $C\alpha$ atomic coordinates, to yield a coarse-grained representation of the protein. Each of the simplices in the ensuing protein structure tessellation objectively identifies at the vertices a quadruplet of structurally nearest neighbor amino acid residues. To ensure only biochemically feasible quadruplet interactions, all protein structure tessellations are modified by the removal of edges longer than 12 angstroms (Figure 2A). Delaunay tessellation implementations and visualizations, and all subsequent data analyses are performed using a

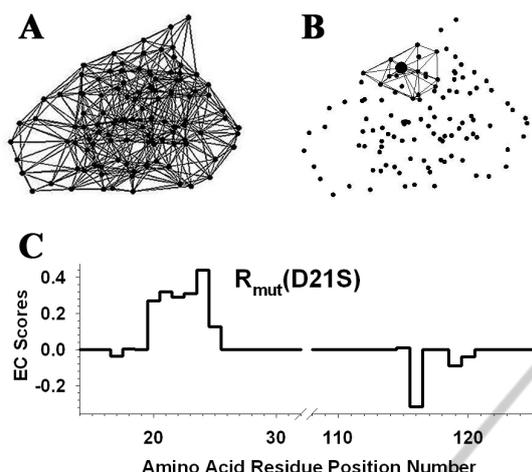


Figure 2: (A) Delaunay tessellation of IL-3 subject to an edge length cutoff of 12 angstroms. (B) Subset of 14 tetrahedral simplices that share the $C\alpha$ vertex of amino acid residue D21 (enlarged relative to the others). There are a total of 11 other vertices among these simplices, and the residues they represent form the neighborhood of D21. (C) R_{mut} vector for the D21S mutant of IL-3. The nonzero EC value at position 21 is the residual score of the D21S mutant, and the other 11 nonzero EC scores identify the D21 neighborhood positions.

combination of Qhull (Barber et al., 1996), Matlab (Version 7.0.1.24704 (R14) Service Pack 1), and an ad hoc suite of Java and Perl codes.

The amino acid building blocks of proteins form a $K = 20$ letter alphabet A . The number of $r = 4$ letter subsets (quadruplets) of A that can be enumerated, assuming permutations of four letters in a quadruplet do not constitute new subsets but that a quadruplet may contain repeats of the same letter, is given by

$$\binom{K+r-1}{r} = \binom{20+4-1}{4} = 8855.$$

These conditions reflect the facts that ordering is not taken into account when four amino acids are identified at the vertices of simplices in protein structure tessellations, and that the same amino acid may appear multiple times in a protein chain and in structurally close proximity to form the vertices of a simplex. Since on average only a few hundred simplices and their respective quadruplets are encountered when a single protein is tessellated, a diverse dataset of 1417 high-resolution protein structures with low sequence and structure similarity was selected for tessellation using the PISCES server (Wang and Dunbrack, 2003) in order to reliably calculate simplicial nearest neighbor relative frequencies of occurrence f_{ijkl} for all 8855 possible

quadruplets (i, j, k, l) in protein structure space. A rate expected by chance for the quadruplets is obtained from the multinomial reference distribution

$$P_{ijkl} = \frac{4!}{\prod_{n=1}^{20} (t_n!)} a_n^{t_n}, \text{ where } \sum_{n=1}^{20} a_n = 1 \text{ and } \sum_{n=1}^{20} t_n = 4.$$

In the above formula, a_n represents the proportion of amino acids of type n in the 1417 tessellated protein structures, and t_n is the number of occurrences of amino acid n in the quadruplet. Through an application of the inverse Boltzmann principle from statistical mechanics (Sippl, 1993), a knowledge based statistical potential of quadruplet interaction is given by the log-likelihood score $s_{ijkl} = \log(f_{ijkl}/p_{ijkl})$, and the collection of 8855 quadruplet types together with their respective scores defines the four-body statistical potential (Carter et al., 2001).

2.3 Computational Mutagenesis

With the Delaunay tessellation of the human IL-3 protein structure, the four-body statistical potential can be used to assign a score to each of the constituent tetrahedral simplices equivalent to that of the amino acid quadruplet identified at its four vertices. Since each amino acid vertex is generally shared by a number of adjacent tetrahedral simplices, the residue participates in multiple nearest neighbor quadruplets. All amino acids represented at the other vertices of these simplices collectively form a *neighborhood* of that shared residue, and any position in a protein structure tessellation rarely has fewer than six neighbors (Figure 2B). Although amino acids positions in the neighborhood are all structurally near their shared residue in 3D Euclidean space, they are often distant from the shared residue in primary sequence. For an amino acid at primary sequence position i in the protein, the *residue environment score* q_i is defined as the sum of scores of all tetrahedral simplices that share its $C\alpha$ vertex (Carter et al., 2001, Zhang et al., 2008).

The environment scores of all amino acids in the native protein can be arranged to form a *3D-ID potential profile* (Bowie et al., 1991) vector $\mathbf{Q}_{wt} = \langle q_1, q_2, q_3, \dots, q_N \rangle$, where the translation $i = i - 13$ has been applied to the residue positions 14 – 125 of the human IL-3 structure, and $N = 112$. A similar profile \mathbf{Q}_{mut} can be obtained for any IL-3 mutant due to a residue substitution at some position j , by first replacing the identity of the amino acid accordingly at the vertex representing position j in the tessellation, and then recalculating all the residue

environment scores. However, the only environment scores that are actually altered occur at the mutated position j and at those that form its neighborhood in the tessellation. The difference $\mathbf{R}_{mut} = \mathbf{Q}_{mut} - \mathbf{Q}_{wt} = \langle EC_1, EC_2, EC_3, \dots, EC_N \rangle$ is a sparse mutant vector that quantifies relative *environmental changes* or perturbations $EC_i = q_{i,mut} - q_{i,wt}$ at every residue position i in the protein due to the mutation (Carter et al., 2001, Zhang et al., 2008). Since the only nonzero EC components of \mathbf{R}_{mut} occur at mutated position j and at positions in its neighborhood, important local effects of a mutation are effectively modelled; however, long-range consequences at structurally distant protein positions are excluded (Figure 2C). We refer to the EC_j component in the vector \mathbf{R}_{mut} at the mutational epicenter j as the mutant *residual score*, due to its significance as a summary measure of the relative change in mutant IL-3 sequence-structure compatibility from that of the native protein. Finally, a *comprehensive mutational profile* (CMP) for IL-3 is a vector obtained by calculating at each position the mean of residual scores associated with all 19 amino acid replacements, where each component is a *CMP score* for the corresponding position (Carter et al., 2001, Zhang et al., 2008).

Conformational changes are effectively accounted for by this computational mutagenesis, both implicitly, through the four-body potential and the perturbation vectors \mathbf{R}_{mut} , and explicitly, due to the use of only coarse-grained $C\alpha$ representations of structures and the fact that Delaunay tessellations are robust to small shifts in the $C\alpha$ coordinates. Hence, a solved structure for every human IL-3 mutant is not required, and tessellation of the only available IL-3 structure suffices. Moreover, these conditions suggest that despite the fact that this IL-3 structure contains 14 residue changes, its tessellation can be used to represent that of the wild type protein by altering the identities of the amino acids at the corresponding vertices to those found in the native IL-3 protein. With these initial alterations, \mathbf{Q}_{wt} can then be computed for wild type IL-3, followed by 3D-1D potential profiles \mathbf{Q}_{mut} and perturbation vectors \mathbf{R}_{mut} for all single residue mutants.

2.4 Random Forest Classification and Evaluation of Performance

A feature vector is generated for each single point human IL-3 mutant whose input attributes (independent variables or predictors) include the mutated position number, the identities of the native and replacement amino acids at the mutated

position, the residual score (i.e., EC score at the mutated position), and the EC scores at the six nearest neighborhood positions ordered nearest to farthest based on 3D Euclidean distance of each neighbor from the mutated position. Next, we include the ordered amino acid identities at the six nearest neighbors as well as their ordered primary sequence locations relative to the mutated position (i.e., difference between neighbor and mutated position numbers). Finally, the following input attributes are added as feature vector components:

- (1) The computed mean volume and mean tetrahedrality for the set of Delaunay simplices that utilize the mutated position as a vertex;
 - (2) The secondary structure {H, helix; C, coil} at the mutated position;
 - (3) Depth {S, surface; U, undersurface; B, buried} at the mutated position (tessellation-based surface accessibility). Surface positions participate as one of three vertices defining a triangular facet for exactly one tetrahedron in the tessellation. Undersurface positions are defined as non-surface positions that share an edge with a surface position. All other positions are buried;
 - (4) A count of the number of simplex edges the mutated position shares with surface positions (zero by definition for buried positions).
- The mutant IL-3 activity class defines the output attribute (dependent variable) associated with each feature vector.

The supervised classification scheme that we employ for this study is an implementation of Leo Breiman's random forest (RF) algorithm (Breiman, 2001), available as part of the WEKA suite of machine learning tools (Frank et al., 2004). The RF algorithm incorporates a *bagging* (bootstrap aggregating) procedure to train an ensemble of classification trees, and predictions are based on a majority vote. The split at each node encountered in the growing trees is based on a fixed-size random subset of the predictor attributes. Also, though all trees are unpruned, the algorithm does not overfit regardless of the number of selected trees. Generally, the RF algorithm performs better than other supervised classification methods (Bordner, 2008, Qi et al., 2006). We fix the adjustable RF parameters in this study at 100 trees, and 5 input attributes are randomly selected for splitting at each tree node.

RF performance on the dataset of IL-3 mutant feature vectors is evaluated by using stratified tenfold cross-validation (10 CV), leave-one-out cross-validation (LOOCV), and stratified random split (66% of dataset for model training and 34% for

testing). Given TP (TN) = total number of correctly predicted “unaffected” or “U” (“affected” or “A”) mutants and FN (FP) = total number of respectively misclassified mutants, the overall accuracy is defined as $Q = (TP + TN) / (TP + FN + FP + TN)$. For the “unaffected” class, $S(U) = \text{sensitivity} = TP / (TP + FN)$ and $P(U) = \text{precision} = TP / (TP + FP)$, while for the “affected” class, $S(A) = TN / (TN + FP)$ and $P(A) = TN / (TN + FN)$. Finally, the balanced error rate (BER) and balanced accuracy rate (BAR), calculated as $BER = 0.5 \times [FN / (FN + TP) + FP / (FP + TN)]$ and $BAR = 1 - BER$, Matthew’s correlation coefficient (MCC), given by

$$MCC = \frac{TP \times TN - FP \times FN}{\sqrt{(TP + FN)(TP + FP)(TN + FN)(TN + FP)}}$$

and area (AUC) under the receiver operating characteristic (ROC) curve provide alternative measures that are especially useful for unbalanced classes. A chi-square test can be applied to assess MCC statistical significance, where the test statistic is given by $\chi^2 = M \times MCC^2$ (M = number of predictions) with one degree of freedom (Baldi et al., 2000). An ROC curve is a plot of true positive rate (sensitivity) versus false positive rate ($1 - \text{specificity}$), and AUC is equivalent to the non-parametric Wilcoxon-Mann-Whitney test of ranks (Fawcett, 2003). An AUC value near 0.5 suggests the trained model will not perform better than random guessing, while a value of 1.0 indicates a perfect classifier.

3 RESULTS AND DISCUSSION

3.1 Human IL-3 Structure-Function Relationships

We begin by generating perturbation vectors \mathbf{R}_{mut} for all 2128 mutants of human IL-3 due to single residue substitutions at positions 14 – 125, from which residual scores are obtained for the 630 mutants of IL-3 with experimentally classified activity. Next, a mean residual score is calculated for the IL-3 mutants in each of the four activity categories and reflects a clear trend (Figure 3A), whereby increasingly detrimental effects on structure (i.e., decreasing mean residual scores) are associated with higher levels of functional impairment (i.e., diminished levels of activity). Moreover, based on six separate t -test applications, a statistically significant difference exists between

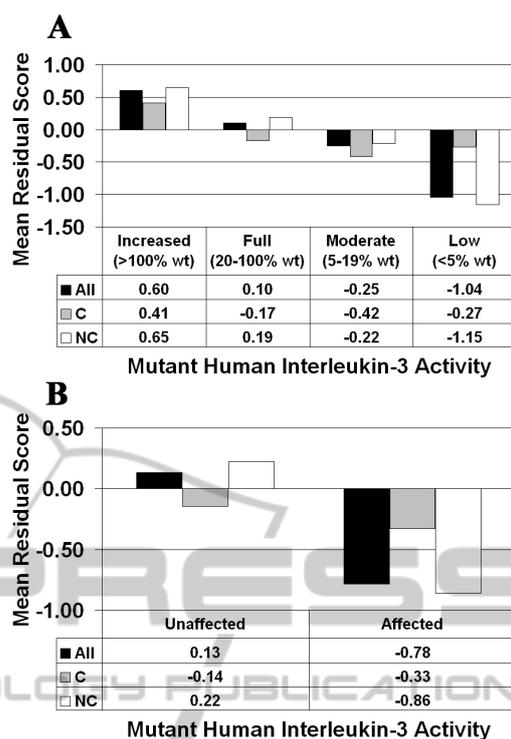


Figure 3: Human IL-3 structure-function correlation based on (A) four mutant activity categories and (B) two functional classes (C / NC = conservative / non-conservative amino acid substitutions).

mean residual scores associated with each pair of activity classes in Figure 3A ($p < 0.05$ in all cases).

The mutants in each class of Figure 3A are further categorized based on whether the residue replacements are conservative (C) or non-conservative (NC) relative to the native amino acids, and mean residual scores are computed for each of these subgroups. With the 20 amino acids clustered into six groups as $\{(A,S,T,G,P), (D,E,N,Q), (R,K,H), (F,Y,W), (V,L,I,M), (C)\}$ based on similarities in physicochemical properties, intraclass residue replacements are considered conservative while interclass substitutions are non-conservative (Dayhoff et al., 1978). Note that the overall trend is driven by NC mutations, since C substitutions minimally impact sequence-structure compatibility regardless of the impact on activity. All results based on four mutant activity categories are identically replicated when we consider the case of two (unaffected / affected) functional classes (Figure 3B), as defined in the Materials and Methods, and the difference in mean residual scores for this class pair is also statistically significant ($p < 0.0001$).

We alternatively consider distribution of the 630 experimental IL-3 mutants in a contingency table

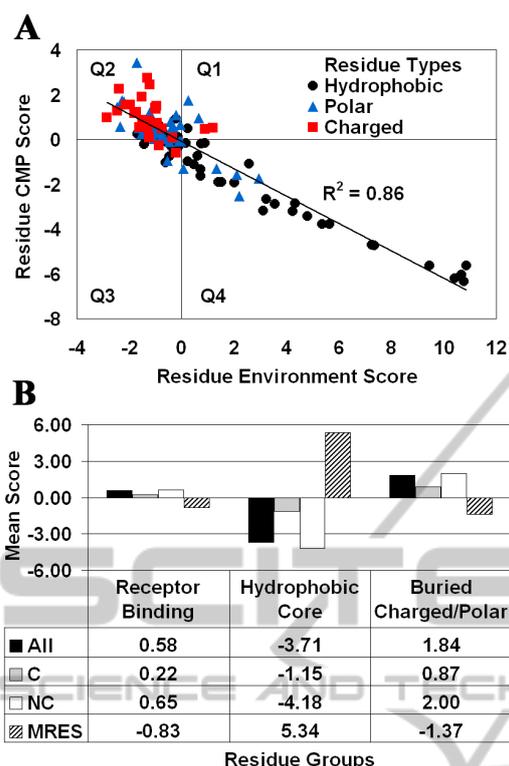


Figure 4: (A) CMP – potential profile correlation plot for IL-3 segregates residues by polarity. (B) Effective discrimination of the functional and structural roles carried out by groups of IL-3 amino acid positions.

based on their activity (out of four classes) as well as their residual scores. Using two categories of residual scores (negative, non-negative), a chi-square test applied to the resulting 4×2 table leads us to reject the null hypothesis that no association exists between activity level and residual scores ($p < 0.01$). Based on four residual score categories (interval boundaries at -0.5, 0, and 0.5), a 4×4 table is generated that yields a similar result ($p < 0.0001$).

3.2 Classification of Human IL-3 Residue Positions

A strong inverse correlation ($R^2 = 0.86$) exists between the CMP profile for human IL-3, obtained by averaging the residual scores of all amino acid replacements at each position, and the 3D-1D potential profile of the protein, which provides an environment score for each position (Figure 4A). By separately averaging residual scores of the non-conservative (NC) and conservative (C) substitutions at each position, a modified NC-CMP profile is computed that is equally inversely correlated with the 3D-1D potential profile ($R^2 = 0.87$) and reflects

the significant contribution of NC substitutions, while a markedly diminished correlation is observed with the modified C-CMP profile ($R^2 = 0.42$). The plot in Figure 4A for 112 residue positions of human IL-3 reveals a clustering according to polarity, with the vast majority of the charged and hydrophobic amino acids occupying quadrants 2 and 4, respectively, and polar residues scattered within a relatively close range of the origin.

In total, 16 residue positions in human IL-3 have been determined to be involved in binding to the IL-3R cell surface receptor: S17, N18, D21, E22, T25, G42, E43, Q45, D46, M49, R94, P96, R108, F113, K116, and E119 (Bagley et al., 1996, Klein et al., 1997). Additionally, based on solvent accessible surface area (<http://curie.utmb.edu/getarea.html>) calculations, the 24 positions most buried in the IL-3 structure consist of 18 hydrophobic residues, with the remaining six amino acids being either charged or polar. We characterize each of these three groups based on both the mean of the residue environment scores (MRES) of the positions in the group, as well as the mean of the mutant residual scores (All, C, NC) for all 19 residue replacements at all positions in the group combined (Figure 4B). Figure 4B clearly shows that our computational characterization effectively distinguishes these functional and structural groups of amino acid positions from one another.

3.3 Prediction of Human IL-3 Activity Changes

As detailed earlier in the Materials and Methods, we first derive feature vectors for all 2128 mutants of human IL-3 due to single residue substitutions at positions 14 – 125, each of which includes only seven of the EC components selected from the corresponding perturbation vector \mathbf{R}_{mut} as well as other predictors. In particular, we first consider feature vectors for the 630 experimental mutants of IL-3, each of which also has an output attribute classifying activity as either “unaffected” or “affected”. Performance of the random forest (RF) algorithm on this training set is evaluated based on running ten iterations each of 10-fold cross-validation (CV) and 66/34 stratified random split, as well as leave-one-out CV (LOOCV), with relatively consistent results across all three testing methods (Table 1). All MCC values are statistically different from zero ($p < 0.0001$), indicating that predictions are notably more correlated with the data compared to random guessing. Table 1 also reveals that our method outperforms by wide margins those of the

SIFT (<http://sift.jcvi.org/>) (Ng and Henikoff, 2006) and SNAP (<http://cubic.bioc.columbia.edu/services/>) (Bromberg and Rost, 2007) state-of-the-art servers that utilize information derived from multiple sequence alignments. Since our model does not incorporate evolutionary information, it serves as an orthogonal approach that is complementary to these other methods.

Table 1: RF model performance and comparisons with other methods.

| Method | Q | MCC | BER | AUC |
|--------------|-----------|-----------|-----------|-----------|
| 10-fold CV* | 0.79±0.01 | 0.54±0.01 | 0.23±0.01 | 0.83±0.01 |
| 66/34 split* | 0.79±0.02 | 0.53±0.05 | 0.24±0.02 | 0.84±0.02 |
| LOOCV | 0.80 | 0.55 | 0.23 | 0.83 |
| SIFT | 0.59 | 0.26 | 0.37 | --- |
| SNAP | 0.68 | 0.33 | 0.33 | --- |

* average over ten iterations.

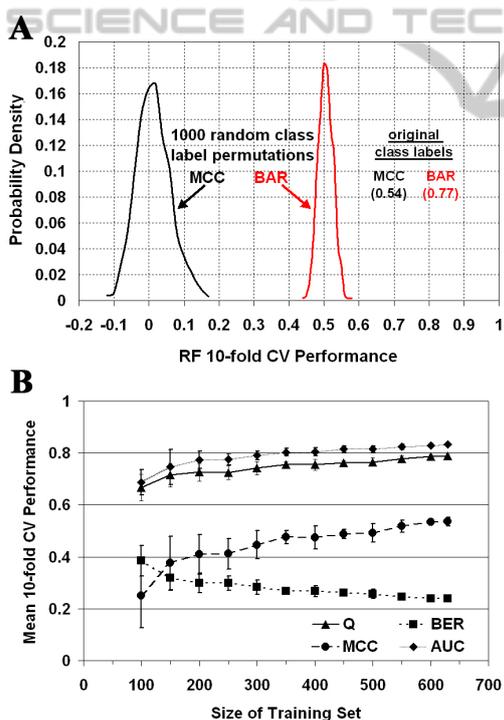


Figure 5: (A) Distribution of 10-fold CV RF prediction performance over 1,000 permutations (random shuffles) of the mutant IL-3 activity class labels. (B) Learning curves. Error bars represent ± 1 std. dev. from the mean.

For a systematic approach to assessing the statistical significance of our results in Table 1, we generate 1,000 activity class label permutations (random shuffles) and calculate the 10-fold CV

performance in each case based on the RF algorithm. The distributions of MCC and BAR accuracy measurements (Figure 5A) are narrowly centered around zero and 0.5, respectively ($MCC = 0.00 \pm 0.04$, $BAR = 0.50 \pm 0.02$), with no permutation accuracy near those obtained using the original arrangement of the class labels (Table 1: $MCC = 0.54 \pm 0.01$, and $BAR = 1 - BER = 0.77 \pm 0.01$), so the p -value for predictive power is less than 0.001.

Next, we undertake a detailed evaluation of the LOOCV predictions on the 630 mutants of human IL-3 in order to assess the strengths and weaknesses of our methodology. In particular, the approach performs best when polar and charged amino acids are replaced with apolar residues, and vice versa, while the least accurate predictions occur with residue substitutions of the same polarity (Table 2). Additionally, although overall accuracy (Q) for surface position mutations appears to surpass that of buried positions (Table 3), contradicting observations noted by other researchers (Bromberg and Rost, 2007, Capriotti et al., 2006), the surface mutations constitute a smaller percentage of the dataset, and their lower correlation coefficient (MCC) suggests those correct predictions may be more biased toward one activity class at the expense of the other. Finally, there are nearly twice as many mutations in helices as there are in coils, and the helix mutation predictions are more accurate.

Table 2: LOOCV prediction performance based on side chain polarities of the native and new amino acids at the mutated position.

| native / new | Polar | | Apolar | | Charged | |
|--------------|-------|------|--------|------|---------|------|
| | Q | MCC | Q | MCC | Q | MCC |
| Polar | 0.71 | 0.16 | 0.85 | 0.47 | 0.80 | 0.24 |
| Apolar | 0.84 | 0.69 | 0.70 | 0.39 | 0.88 | 0.76 |
| Charged | 0.83 | 0.65 | 0.90 | 0.80 | 0.59 | 0.18 |

Table 3: LOOCV prediction performance based on depth and secondary structure.

| Depth | Q | MCC | % | |
|---------------------|------|------|------|----|
| Buried | 0.78 | 0.56 | 42 | |
| Undersurface | 0.77 | 0.50 | 27 | |
| Surface | 0.84 | 0.48 | 31 | |
| Secondary Structure | | Q | MCC | % |
| Helix | | 0.80 | 0.58 | 64 |
| Coil | | 0.79 | 0.48 | 36 |

% refers to the proportion of IL-3 mutants in the dataset.

In order to assess the influence of dataset size on trained RF model performance, learning curves are provided in Figure 5B. We begin by applying RF learning and 10-fold CV to each of 10 stratified

random samples of 100 dataset mutants, where each subset is selected from among all 630 experimental human IL-3 activity mutants, and mean performance and standard deviation (std. dev.) is calculated. Subsequent iterations involve incrementing by 50 mutants the size of the sampled datasets. The learning curves do not appear to reach plateaus as they approach the full dataset size of 630 mutants, suggesting that the current RF model may be improved upon by enlarging the training set through possible future availability of experimental activity data for additional IL-3 mutants.

The *CfsSubsetEval* attribute evaluator program in WEKA is a tool for identifying the most influential feature vector attributes (Frank et al., 2004). The method evaluates various subsets of the attributes for how highly correlated the predictors in each subset are with the unaffected / affected activity classes while also displaying low intercorrelation with one another. The *BestFirst* search program in WEKA is concurrently used to select the subsets for evaluation based on a greedy hill-climbing approach, whereby starting with a random selection of attributes, a bi-directional search ensues in which all possible additions or deletions of single attributes are examined at each step (Frank et al., 2004). The procedure is augmented with backtracking, whereby a maximum of five consecutive, non-improving attributes are allowed. The following ten attributes are identified as the most highly ranked predictors: position number; residual score; EC scores at the second, third, and fifth nearest neighbors; primary sequence locations of the fourth and sixth nearest neighbors relative to the mutated position; amino acid identity at the sixth nearest neighbor to the mutated position; mean volume; and mean tetrahedrality. These ten attributes span the diversity of predictors considered in this study, underscoring the importance of the collective set of features.

Finally as an important practical application, we employ the RF model learned from the entire training set of 630 mutants of human IL-3 in order to predict the unaffected / affected class memberships of all remaining 1498 uncharacterized single residue IL-3 mutants. In particular, we form a test set that contains feature vector input attributes for the 1498 mutants of IL-3 that remain to be predicted, each of which has an unknown unaffected / affected activity class output attribute. Based on the signals encoded by the input attributes of their feature vectors, the

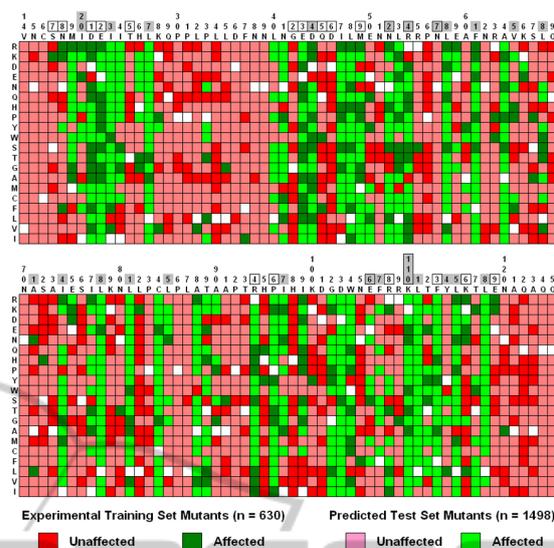


Figure 6: Human IL-3 mutational array (red = unaffected, green = affected, white = self-substitutions; darker colors = experimental, lighter = predicted; boxed numbers = receptor binding, shaded = hydrophobic core, both shaded and boxed = buried charged / polar).

RF model generates a class prediction for every IL-3 mutant in the test set. All experimental and predicted IL-3 mutants are pooled into the array shown in Figure 6, which concisely summarizes overall protein mutational patterns. Columns represent residue positions in IL-3, and rows represent the 20 possible amino acid replacements, arranged from top to bottom in order of increasing hydrophobicity (Kyte and Doolittle, 1982). Notably, at nearly all receptor-binding positions for which a number of amino acid substitutions are known to affect activity, predictions match experimental IL-3 mutant data.

4 CONCLUSIONS

This study demonstrates the utility of a computational mutagenesis methodology, based on implementations of a four-body potential and the Delaunay tessellation of protein structure, for modeling single residue replacements in the human interleukin-3 (IL-3) cytokine. For each IL-3 mutant, the approach quantifies environmental perturbation at the mutated position (i.e., the residual score) and at all positions in its neighborhood, as defined by the tessellation of the IL-3 protein structure. Published experimental data include relative changes in cell proliferation activity for 630 single residue substitutions of IL-3, representing nearly 30% of all such mutants in the protein. An IL-3 structure-

function relationship is elucidated with this collection of mutants by comparing their residual scores (measures of relative changes to sequence-structure compatibility) with their relative activity changes (measures of relative functional changes). More generally, residual scores are also useful for naturally clustering IL-3 amino acid positions based on their polarity, as well as for distinguishing residue groups based on their functional or structural roles. The experimental IL-3 mutants are subsequently represented as feature vectors, with input attributes that include the residual score, ordered perturbation scores for the six structurally closest positions in the local neighborhood of the mutated position, and additional components based on sequence and structure, as well as an activity category (unaffected / affected) output attribute. This collection of feature vectors is used to train a random forest classifier, which displays up to 80% accuracy for mutant IL-3 activity prediction and outperforms other well-known methods. To assist researchers in prioritizing future IL-3 mutagenesis experiments, activity predictions based on the trained model are provided for all 1498 unexplored single residue IL-3 mutants.

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