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RETROVIRAL PROTEASES VIEWED THROUGH THE RESONANT RECOGNITION MODEL*

RETROVIRALNE PROTEAZE RAZMOTRENE SA MODELOM REZONANTNOG PREPOZNAVANJA*

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Abstract

We have applied Resonant Recognition Model (RRM) to computationally analyze HIV proteases with the aim to predict functionally important amino acids. We compared predicted hot spots and simulated single mutations with experimentally tested mutations and we found significant correlation. In this way we also predicted a number of key amino acids that have not yet been experimentally tested and as such could bring new mutants with desired functionality.

INTRODUCTION

Human Immunodeficiency Virus (HIV) protease (PR) is one of the three enzymes found in the HIV virus. This enzyme cleaves newly synthesized polyproteins to create the mature protein components of an infectious HIV virion. Without effective HIV protease, HIV infection is not possible. This explains why protease inhibitors are an important class of antiretroviral drugs: by year 2007, 10 out of 25 anti-HIV agents licensed for clinical use were protease inhibitors (saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, lopinavir, atazanavir, fosamprenavir, tipranavir and darunavir) [1].

PRs, however, are highly adaptable and mutating quickly into variants with several hundred times less affinity for the inhibitor and still retaining significant proteolytic activity.

HIV protease has been described in detail in the framework of classical molecular biology approaches, as well as from the viewpoint of bioinformatics [2-4]. As result, the three-dimensional structure, the active centre as well as the minimal core of amino acids that has to remain unchanged in any viable enzyme have been determined.

On the other hand, a great deal of experimental data about the effect of different single-point and multiple mutations upon catalytic activity and inhibitor affinity has been published [5-21].

Taking into account the formidable difficulties associated with HIV/AIDS therapy it is necessary to explore the new innovative approaches. One of such innovative approaches is Resonant Recognition Model (RRM) [22-31].

This approach has been applied to the study of many enzymes and proteins and has been capable of theoretically predicting aspects of the functional “key” amino acids in a sequence (hot spots) as well as the design of new protein inhibitors.

As early as 1994, Cosic used the Resonant Recognition Model for the characterization of HIV envelope proteins [26], which are important participants in the process of recognition of CD4 cells by the HIV virion [1- 32].

We hypothesize that the RRM approach can be also useful for characterizing important aspects of HIV protease. It is not excluded that, given its sound theoretical framework, some unknown facts about HIV_PR can be understood and the way for new approaches in HIV/AIDS therapy can be paved.

In this work we identify the main RRM characteristic resonant frequencies of retroviral proteases. The „key" amino acids (hot spots) are identified through direct prediction as well as through the effect of some point mutations. Finally, data are compared with experimental data reported in literature and significant correlation was found.

METHODS

Resonant Recognition Model

The RRM is a physico-mathematical approach that interprets protein as univariate numerical sequences upon which digital signal processing methods are applied [26-29]. The RRM postulates that protein (DNA) interactions entail a mechanism of resonant energy transfer between the interacting molecules at the frequency specific for each observed function/interaction. Within this model, the protein primary structure is represented as a numerical series by assigning to each amino acid in the sequence a physical parameter value relevant to the protein's biological activity. Through using the RRM, it has been hypothesized that there is a significant correlation between spectra of the numerical presentation of amino acids and their biological activity. It has been found that proteins with the same biological function have a common frequency component in their numerical spectra. This frequency is considered to be a characteristic feature of a protein's biological function or interaction. The RRM procedure involves two stages of calculations.

First, the original amino acid sequence is transformed into a numerical sequence by assigning to each amino acid a particular value of the physical parameter relevant to a protein's biological function. Here, the energy of delocalized electrons (calculated as the electron–ion interaction pseudopotential), EIIP [29] of each amino acid residue is used. The EIIP parameter describes the average energy states of all valence electrons in a particular amino acid. The EIIP values for each amino acid were calculated from the general model of pseudo-potentials:

$$\langle k+q|wk|\rangle = 0.25\frac{Z\sin(1.04*\pi z)}{2\pi}$$

where q is a change of momentum k of the delocalized electron in the interaction with potential w, and

$$Z = \frac{\sum_i Z_i}{N}$$

being Z_i is the number of valence electrons of the i-th atom of each amino acid and N is the total number of atoms in the amino acid. Thus, the resulting numerical series represents the distribution of the free electron energies along the protein. The numerical sequences obtained are analyzed using Fourier Transform (FFT) to extract information pertinent to the biological function. As the average distance between amino acid residues in a protein sequence is about 3.8 Å, it can be assumed that the points in the numerical sequence derived are equidistant (the analog of an equisampled time series). For further numerical analysis, the distance between points in these numerical sequences is set at an arbitrary value: d=1. Peak frequencies in the amplitude cross-spectral function define common frequency components of the two sequences analyzed. In order to determine the common frequency components for a group of protein sequences, we have calculated the values of multiple cross-spectral function coefficients, Mn which is defined as follows:

$$|Mn|=|X1n|.|X2n|...|XMn| \quad n=1,2,...,N/2$$

where n is the number of cross correlated proteins, Mn represents the n-th spectral component in the cross-spectral function and Xk,n is the n-th spectral component of the k-th protein. Peak frequencies in such a multiple cross-spectral function denote common frequency components for all the sequences analyzed. The multiple cross-spectral functions for a large group of sequences with the same biological function has been named 'consensus spectrum'. The presence of a distinct peak frequency in a consensus spectrum implies that all of the analyzed sequences within the group have one frequency component in common. This frequency is related to the biological function provided the following criteria are met:

(1) One peak only exists for a group of protein sequences sharing the same biological function.

(2) No significant peak exists for biologically unrelated protein sequences.

(3) Peak frequencies are different for different biological functions.

In previous research [22, 33-38] the above criteria have been implemented, and the following fundamental conclusion was drawn: each specific biological function of a given protein or DNA is characterized by a single frequency. Our previous research showed that proteins with the same biological function have a common frequency in their numerical spectra, and each specific biological function of a protein or regulatory DNA sequence(s) is characterized by a single frequency [30]. The results of previous studies with a number of different protein families revealed that proteins and their interacting targets (receptors, binding proteins and inhibitors) display the same characteristic frequency in their interactions. However, it is obvious that one protein can participate in more than one biological process, i.e. revealing more than one biological function. Therefore, it has been postulated that the RRM frequency characterizes a particular biological process of interaction between selected biomolecules. Thus, the RRM characteristic frequencies represent a protein's general functions as well as the mutual recognition between a particular protein and its target (receptor, ligand, etc.). As this recognition arises from the matching of periodicities within the distribution of energies of free electrons along the interacting proteins, it can be regarded as the resonant recognition.

Once the characteristic frequency for the particular biological function or interaction is determined, it becomes possible to identify the individual "hot spot" amino acids that contributed most to this specific characteristic frequency and thus, possibly to the observed biological behavior of the protein. These „key" amino acids are found to be clustered in and around a protein's active sites.

In this work we applied RRM to the following sequences which have been downloaded from SwissProt databank:

>sp|P27973|512-612 Simian Immunodeficiency Virus (MAL)
>sp|P05895|516-616 Simian Immunodeficiency Virus (TYD)
>sp|Q02836|505-607 Simian Immunodeficiency Virus (AGM)
>sp|P12502|502-597 Simian Immunodeficiency Virus (SM)
>sp|P05897|501-596 Simian Immunodeficiency Virus (KBW)
>sp|P24107|513-611 Human Immunodeficiency Virus (CAM2)
>sp|P03366|501-599 Human Immunodeficiency Virus (BH1D)
>sp|P05961|495-593 Human Immunodeficiency Virus (MN)
>sp|P04588|494-592 Human Immunodeficiency Virus (MAL)
>sp|P26315|1-124 Bird (Endogenous Retroviral sequence)
>sp|P04024|1-314 Monkey (Endogenous Retroviral sequence)
>sp|P11365|690-765 Mouse (Endogenous retroviral Sequence)
>sp|P10265|21-96 Human (Endogenous retroviral sequence)
>sp|P0CT34|266-342(q05654) Schizosaccharomyces pombe (Endogenous Retroviral sequence)
>sp|P35956|1-135 Visna virus
>sp|P23427|1-139 Visna virus
>sp|P16088|1-154 Feline Immunodeficiency Virus
>sp|P19560| [BIV]Bovine Immunodeficiency Virus

Hot spots determination

By identifying the characteristic frequency of a particular protein, it is possible to predict which amino acids in the sequence predominantly contribute to the frequency and consequently to the observed function [23, 26, 28, 29]. Since the characteristic frequency correlates with the biological function, the positions of the amino acids that are most affected by the change of amplitude at the particular frequency can be defined as hot spots for the corresponding biological function.

The strategy for this prediction includes the following steps:

1. The unique characteristic frequency for the specific biological function is determined by multiple cross-spectral analyses for the group of sequences with the corresponding biological function.
2. The amplitude is altered at this characteristic frequency in the particular numerical spectrum. The criterion used for identifying the critical characteristic frequency change is the minimum number of hot spot amino acids that are least sensitive to further changes in the amplitude of the characteristic frequency.
3. A numerical sequence from the modified spectrum is derived using Inverse Fourier Transform (IFT). It is known that a change in amplitude at one frequency in the spectrum causes changes at each point in the numerical sequence. Thus, a new numerical series is obtained where each point is different from those in the original series. Detecting the amino acids corresponding to each element of this new numerical sequence can then be achieved using tabulated values of the EIIP or other appropriate amino acid parameters.

The amino acids in the new sequence that differ from the original ones reside at the points most contributing to the frequency. These hot spots are related to this frequency and to the corresponding biological function.

Mutation simulations

Point mutations:

To theoretically explore the contribution of each amino acid to the amplitude of a resonant peak at a given position the given amino acid was changed to leucine (EIIP=0) or to aspartic acid (EIIP=0.12630). If the EIIP of the given amino acid is lower than 0.07 the mutated amino acid is aspartic acid, otherwise, if the original amino acid's EIIP is higher than 0.07 the mutated amino acid is leucine (EIIP=0.0). Relative amplitude change (no change=1.0) is estimated for each amino acid at the given resonant position.

Other mutations:

To compare experimentally reported results with theoretical predictions, peak amplitudes were obtained from the reported mutated proteases.

RESULTS

Resonant frequency identification

The cross-spectrum for the 18 retroviral protease sequences is presented in Figure 1. As apparent, a very prominent peak appears at f=0.0586±0.004.

With the four HIV proteases, the most prominent peak appears at f=0.1797±0.004 as presented in Figure 2.

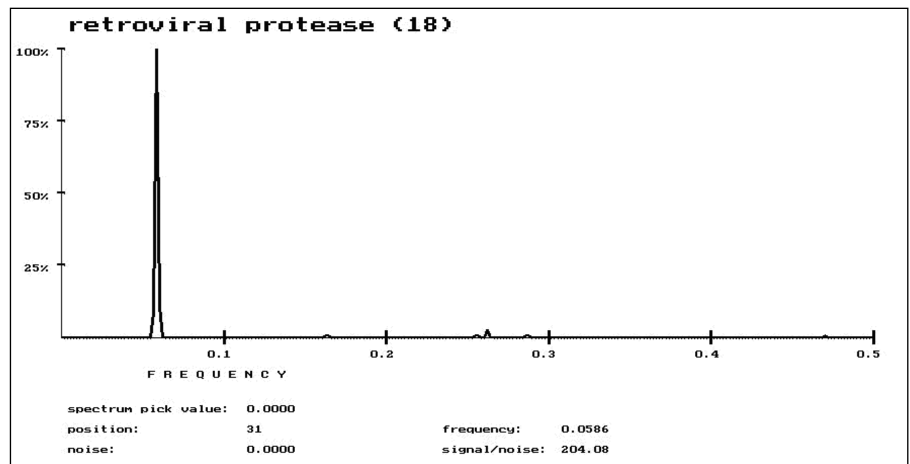

***Figure 1**: Consensus RRM spectrum of 18 retroviral protease showing the prominent common frequency at f= 0.0586±0.004.*

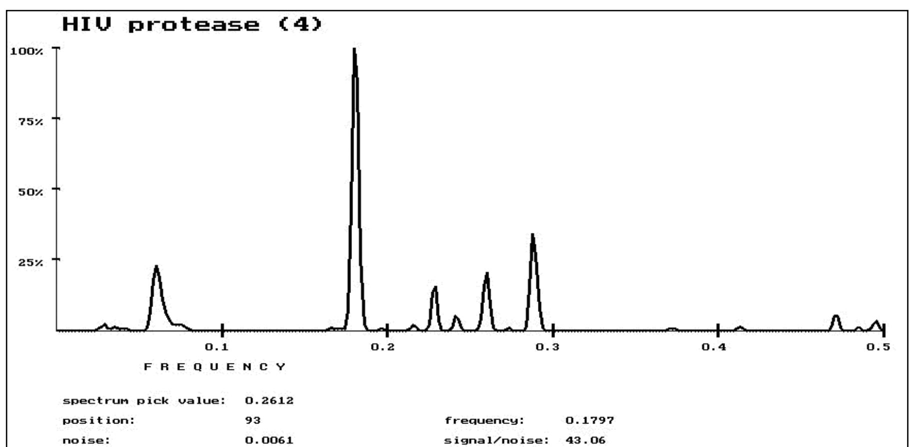

***Figure 2**: Consensus RRM spectrum of 4 HIV protease showing the most prominent peak at f= 0.1797±0.004.*

Combining Simian (SIV) and Human (HIV) proteases revealed two peaks at 0.0586+0.004 and 0.1797±0.004 as presented in Figure 3, but SIV proteases alone exhibited one peak at 0.0586±0.004.

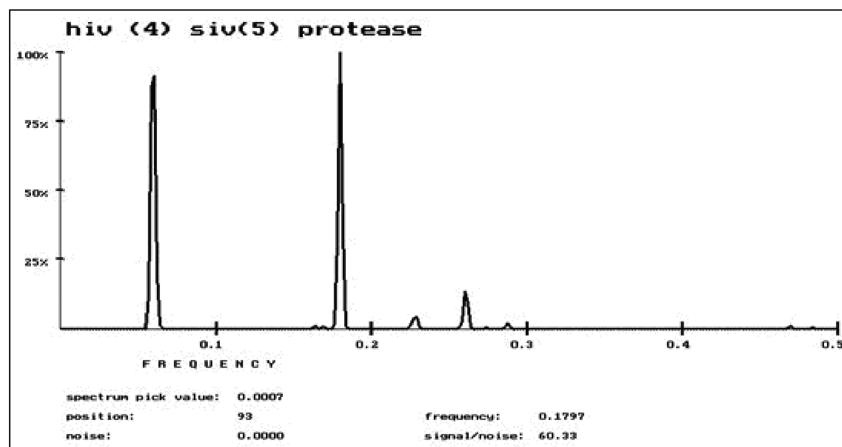

***Figure 3**: Consensus RRM spectrum of 4 HIV protease and 5 SIV protease showing two prominent peaks at 0.0586+ 0.004 and 0.1797+ 0.004.*

Thus, the presence of prominent peaks at f=0.0586±0.004 and f=0.1797±0.004 appear to characterize different subsets of retroviral proteases. Particularly, the peak at 0.0586±0.004 is shared by all retroviral proteases, and the peak at 0.1797±0.004 seems to be more specific for HIV and SIV proteases together.

As an interesting fact, in 1994 Cosic found a peak at 0.1855±0.004 for HIV envelope proteins. This frequency could be considered overlapping with the frequency obtained here for HIV proteases (within the digitalization error). Thus the peak at 0.1797±0.004 seems to be associated to specific HIV activity. In following sections we will try to provide support for this idea [26].

Hot spot analysis

Hot spot analysis as described above was applied to HIV Cam2 protease using both 0.0586±0.004 and 0.1797±0.004 frequencies. The analysis is performed for both increasing and decreasing the amplitude at these two frequencies and the six most relevant mutations are chosen for each of changes. Mutations related to decreasing the amplitude are supposed to be related to decreasing of the relevant function while mutations related to increase of the amplitude are supposed to be related to increase of the related biological function. The results are presented in the Table 1 below.

***Table 1:** Representing "hot spot" amino acid predictions for two different relevant frequencies 0.0586±0.004 and 0.1797±0.004 for increase and decrease of amplitude on these frequencies.*

Frequency	% of change	Predicted mutation agreement with experiment	Predicted mutation agreement with experiment	Predicted mutation agreement with experiment	Predicted mutation agreement with experiment	Predicted mutation agreement with experiment	Predicted mutation agreement with experiment	Expected effect
0.0586±0.004	-3.5	17G	35G yes	49G yes	51G yes	52G	68G	Decrease of proteolytic activity
0.0586±0.004	+5.6	27G	39G	53F yes	78G	85F	94G	Increase of proteolytic activity
0.1797±0.004	-3.5	17G	27G	39G	49G yes	77T	94G	Decrease of HIV related activity
0.1797±0.004	+4.8	35G yes	43S	51G yes	52G	68G	86G yes	Increase of HIV related activity

As it can been seen from the table above there are some agreements with already experimentally proved "key" mutations. However, there are number of predictions which still need to be experimentally tested and might have a significant effect on protease activity.

Effect of „strong" mutations at each amino acid

As described in the „methods" section, we simulated mutations that change an amino acid with low EIIP value for an amino acid with high EIIP value. Even when this does not correspond to the whole repertoire of possible mutations, it can give an exploratory idea of the effect of mutations upon the resonant peak amplitude. The effect of „strong" mutations at each of the amino acid upon amplitude at f=0.0586±0.004 is presented in Figure 4.

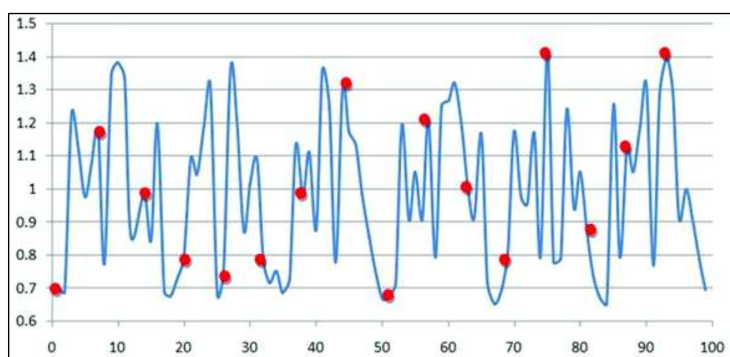

***Figure 4**: The effect of „strong" mutations at each of the amino acid upon amplitude at f=0.0586±0.004.*

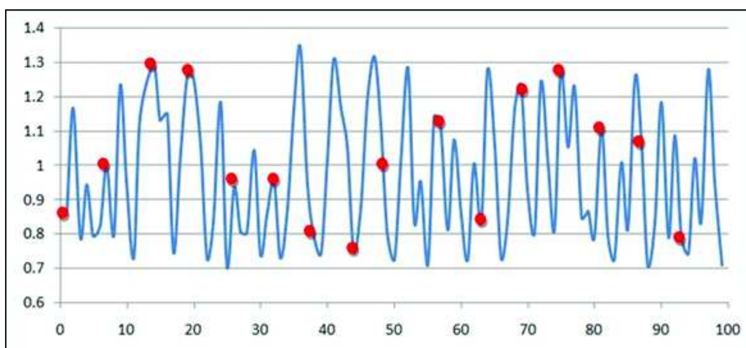

***Figure 5**: The effect of „strong" mutations at each of the amino acid upon amplitude at f=0.1797±0.004.*

As noticed, amino acid single substitutions can lead to either increases or decreases in the peak's amplitude at the frequency associated to protease activity. Indeed, 54 percent of mutations reduce the amplitude of the resonant peak at 0.0586±0.004.

We hypothesize that this can be relevant to the high adaptability of proteases under the pressure of inhibitors: a repertoire of mutations where combination of proteolytic activity and proteolytic inhibition will allow reduced affinity to inhibitors with no severe compromises for proteolytic activity.

In Figure 5 a similar result is shown for the effect on amplitude at peak at f=0.1797±0.004. Similarly, 48 percent of mutations reduce the amplitude of the resonant peak at 0.1797±0.004.

Comparing theoretical predictions with experimental reports

We found in literature indications on how at some point mutations affect the catalytic activity. We compared these results from theoretical predictions from Figure 4. and these comparisons are summarized in Table 2.

Thus, from 28 documented single-point mutations, correspondence between theoretical prediction and catalytic activity has been confirmed for 26 of them (93%). This gives strong support to the validity of RRM for the study of HIV proteases.

CONCLUSION

We have applied here Resonant Recognition Model to analyze HIV proteases with the aim to find out their characteristic function related features (frequencies) and consequently to predict and analyze „key" amino acids and related mutations. Our study revealed that HIV protease exhibits two RRM resonant peaks, one peak at f=0.0586±0.004 is likely to be associated to proteolytic activity, whereas the peak at f=0.1797±0.004 might be associated to HIV specific interactions of the protease. This is supported by the similarity between this frequency and that reported by Cosic (1994) [26] for HIV envelope proteins. In favor of the former are data from activity reported for different point mutations in Table 2.

Using these RRM characteristic frequencies then we were able to investigate influence of particular amino acids to each of these characteristic frequencies. This can lead to functional mutations that can either increase or decrease the

***Table 2**: Summary of comparisons between "strong" mutations and experimentally tested mutations. Mutations in red denote decreased activity, mutations in green denote increase activity, while mutations in purple denote reduced catalytic activity.*

AA position	Amplitude change (no change=1)	Experimental report (reference)	Agreement
84	0.6524248	Catalytic efficiency reduced [5]	Yes
50	0.6677426	Decreased Catalytic efficiency [6]	Yes
51	0.6765125	Decreased catalytic efficiency [7]	Yes
25	0.6780	Inactive [2]	Yes
35	0.6887	May reduce catalytic efficacy [8]	Yes
82	0.7360055	Lower catalytic activity [9]	Yes
26	0.7504616	Crucial for catalysis [10]	Yes
49	0.7536639	Inhibited HIV infectivity [11]	Yes
26	0.7504616	Inactive [12]	Yes
8	0.773679	Lower activity [13]	Yes
76	0.7803	Unstable and slow auto processing [14]	Yes
32	0.7871999	Kcat reduced [5]	Yes
86	0.7956613	Defective catalytic activity [10]	Yes
48	0.8565207	From 50 to 80% of wild type [13]	Yes
54	0.9074061	Decreased catalytic efficiency [7]	Yes
A28S	0.9738727	Catalytic activity reduced [39]	Yes
10	0.9786019	Mutation appears to be silent in growth characteristics [16]	Yes
63	1.0005	As fit as Wild type [17]	Yes
88	1.0519193	Lower catalytic activity, slower viral growth [16]	No
80	1.0533545	Comparable to the wild protease [18]	Yes
87	1.121996	Reduced catalytic activity [10]	No
46	1.1320	Higher activity than wild type [13]	Yes
37	1.1349886	Catalytic efficiency falls [12]	Yes?
7	1.170872	Enhanced [19]	Yes
45	1.1751233	Up to 110% activity increase [13]	Yes
90	1.3142611	Relative Kcat between 1.2 and 1.6 [20]	Yes
55	1.0519193	A role in enhancing viral replication [21]	Yes
53	1.1949737	Catalytic efficiency increased [7]	Yes

P01 Q02 F03 S04 L05 W06 K07 R08 P09 V10 V11 T12 A13 H14 I15 E16 G17 Q18 P19 V20
P01 Q02 F03 S04 L05 W06 K07 R08 P09 V10 V11 T12 A13 H14 I15 E16 G17 Q18 P19 V20

E21 V22 L23 L24 D25 T26 G27 A28 D29 D30 S31 I32 V33 A34 G35 I36 E37 L38 G39 S40
E21 V22 L23 L24 D25 T26 G27 A28 D29 D30 S31 I32 V33 A34 G35 I36 E37 L38 G39 S40

N41 Y42 S43 P44 K45 I46 V47 G48 G49 I50 G51 G52 F53 I54 N55 T56 K57 E58 Y59 K60
N41 Y42 S43 P44 K45 I46 V47 G48 G49 I50 G51 G52 F53 I54 N55 T56 K57 E58 Y59 K60

N61 V62 E63 I64 E65 V66 L67 G68 K69 R70 V71 R72 A73 T74 I75 M76 T77 G78 D79 T80
N61 V62 E63 I64 E65 V66 L67 G68 K69 R70 V71 R72 A73 T74 I75 M76 T77 G78 D79 T80

P81 I82 N83 I84 F85 G86 R87 N88 I89 L90 T91 A92 L93 G94 M95 S96 L97 N98 L99
P81 I82 N83 I84 F85 G86 R87 N88 I89 L90 T91 A92 L93 G94 M95 S96 L97 N98 L99

***Figure 6**: The upper line is predictions and experimental single spot mutations kept in colors red, green and purple as per Table 2. In the lower line are hot spot predictions as per Table 1. all highlighted in yellow.*

proteolytic and/or HIV specific activity. We have shown that there is significant correlation between already experimentally tested mutations and characteristic frequency peak values. These results are summarized in the Figure 6. below where single point mutations both predicted and experimentally tested as per table are highlighted in HIV Cam2 sequence (first line). Hot spots predicted with inverse RRM where highlighted in yellow in second line. It can be observed that all these predictions are clustered around conserved regions in the HIV Cam2 sequence.

We have shown here once again that Resonant Recognition Model can identify functionally relevant features (frequencies) within the protein primary structure and consequently successfully predict functional mutations. Such computational procedure can significantly lower the time and expense for design of new, mutated proteins and peptides with the desired function.

Acknowledgment

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Sažetak

U ovom radu smo primenili Model Resonantnog Prepoznavanja (RRM) u kompjuterskoj analizi HIV proteaza sa ciljem da predvidimo amino kiseline koje su važne za njihovu biološku funkciju. Uporedili smo “hot spot” i simulirane predikcije sa eksperimentalno testiranim mutacijama i dobili značajno slaganje. Takođe smo predvideli i amino kiseline koje još nisu eksperimentalno testirane, a mogle bi da budu značajne za moguće funkcionalne mutacije.

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