

# Variation in site entropy explains differences in structure-sequence relationships of proteins

Eleisha L. Jackson<sup>2\*</sup>, Amir Shahmoradi<sup>1\*</sup>, Claus O. Wilke<sup>2\*\*</sup>

May 28, 2015

<sup>1</sup> Department of Physics, The University of Texas at Austin, Austin, TX 78712, USA

<sup>2</sup> Institute of Cellular and Molecular Biology, Center for Computational Biology and Bioinformatics, and Department of Integrative Biology, The University of Texas at Austin, Austin, Texas, 78712 USA

\*These authors contributed equally.

\*\*Corresponding author

Email: wilke@austin.utexas.edu

Phone: +1 512 232 2459

Manuscript type: research article

Keywords: protein evolution, relative solvent accessibility, site variability

## Abstract

Recent work has shown that structural properties are capable of predicting site-specific sequence variability for a given protein. However, the strength and significance of these structure-sequence relations appear to vary widely among different proteins, with absolute correlation strengths ranging from 0.1 to 0.8.

# 1 Introduction

Proteins are subject to a number of biophysical and functional constraints (Scherrer et al., 2012; Wilke and Drummond, 2010). These constraints result site-specific patterns of sequence variability within a protein. Recently several site-specific structural properties that can explain patterns of sequence variability in proteins have been identified. One of the earliest examples is Relative Site Accessibility (RSA). Franzosa and Xia (2009) identified RSA as the strong predictor of evolutionary rate and found that residues that are buried in the core of proteins tend to be more conserved than exposed residues close to the surface of the protein. In their analysis they considered the ability of both RSA and various definitions of residue packing density to predict evolutionary rate. They found that RSA and evolutionary rate shared a significant linear relationship. Afterwards, several other works (Ramsey et al., 2011; Scherrer et al., 2012) also found that RSA as a very significant predictor of evolutionary rate and found this linear relationship as well. However, these papers all have the same flaw. During the course of their analysis they binned the protein sites and average over all sites within a bin when looking at the trend of RSA. This process may have produce artifacts that account for this strong linear trend.

Recently, Yeh et al. (2014) performed a similar analysis on a series of enzyme monomer proteins and found that packing density, as defined by CN and WCN (Liao et al., 2005; Yeh et al., 2014; Huang et al., 2014), was the strongest determinant of site variability. A year later, Shahmoradi et al. (2014) also performed a site-wise analysis on a series of viral proteins. In this analysis they found that RSA had the strongest correlation with site variability as opposed to local packing density. Additionally the effect seen between CN and WCN was of a much smaller magnitude as compared to (Yeh et al., 2014). Therefore the relationship between local packing density, RSA and site-specific measures of sequence variability is not well understood. Here we attempt to reconcile the work done in this area. We find that site variability is the primary determinant of the strength of structure-sequence relationships and some differences in previous work can be explained in terms of differing levels of site variability.

## 2 Materials and Methods

### Structures, sequences, and measures of sequence properties

The results presented in this work are based on two datasets. The first is a dataset of 209 monomeric enzymes taken from Huang et al. (2014), originally from Yeh et al. (2014). The original dataset was comprised of 213 proteins but we removed four of the proteins (PDB IDs: 1BBS, 1BS0, 1DIN, 2HPL) that had did not have data at insertion sites. Briefly, these proteins are all enzyme monomers randomly picked from the Catalytic Site Atlas 2.2.11 (Porter et al., 2004) with protein sizes in the sample ranging from 95 to 1287 residues in length. For each structure we had a corresponding alignment of up to 300 homologous sequences. The second dataset was taken from Shahmoradi et al. (2014) and is comprised of nine viral proteins. The viral proteins range from 122 - 557 residues in length and each

structure is accompanied by a sequence alignment of up to 2362 homologous sequences. Sequence alignments for both datasets were constructed by aligning the amino-acid sequences using the alignment software MAFFT (Kato et al., 2002, 2005), specifying the auto flag to select the optimal algorithm for the given data set. The alignments were then used to calculate the site-specific measures of sequence variability for each individual protein in both datasets. To do so, we relied on two independent methods of measuring sequence variability. First, we calculated the Shannon entropy ( $H_i$ ) – the sequence entropy at each alignment column  $i$ :

$$H_i = - \sum_j P_{ij} \ln P_{ij} \quad (1)$$

where  $P_{ij}$  is the relative frequency of amino acid  $j$  at position  $i$  in the alignment. The sequence entropy is a measure of variability at each site. We also calculated a measure of site-specific evolutionary rate for each protein using software Rate4site. First the Maximum Likelihood phylogenetic trees were inferred with RAxML, using the LG substitution matrix and the CAT model of rate heterogeneity (Stamatakis, 2006, 2014). For each structure, we then used the respective sequence alignment and phylogenetic tree to infer site-specific substitution rates with Rate4Site using the empirical Bayesian method and the JTT model of sequence evolution (Mayrose et al., 2004).

## Calculation of Structural Properties

In our analysis we used two types of measures of local packing density used in previous studies: Contact Number (CN) and Weighted Contact Number (WCN). For the purposes of our comparison between the two datasets of interest we used the CN and WCN for the enzyme proteins calculated in Huang et al. (2014) and the CN and WCN values for the viral proteins from Shahmoradi et al. (2014). In both of these works and in Yeh et al. (2014), WCN and CN are both defined the same. Contact Number is defined as the number of  $C_\alpha$  within a pre-redefined radius,  $r_0$ . In this case,  $r_0 = 13$  as in the previous papers. Weighted Contact Number for a residue,  $i$ , is defined as in (Liao et al 2005, Huang et al 2014), as:

$$\text{WCN}_i = \sum_{i \neq j}^N \frac{1}{r_{ij}^2} \quad (2)$$

where  $r_j$  is the length between the  $C_\alpha$  of residue  $i$  and residue  $j$  in a protein of length  $N$  (Yeh et al., 2014).

We used DSSP (Kabsch and Sander, 1983) to calculate the Accessible Surface Area (ASA) for each site. We then normalized the ASA for each site by the theoretical maximum solvent accessibility values of Tien et al. (2013) to obtain the Relative Solvent Accessibility (RSA) for all individual sites in all proteins.

All data and analysis scripts required to reproduce the work are publicly available to view and download at [https://github.com/wilkelab/rate\\_variability\\_variation](https://github.com/wilkelab/rate_variability_variation).

### 3 Results

We calculate the strength between the structural properties and site variability by calculating the Spearman correlation,  $\rho$ , between the structural property (solvent exposure and local packing density) and site variability, as measured by either evolutionary rate or site entropy. On average the correlations between CN and entropy are larger in absolute magnitude in the enzyme proteins as compared to viral proteins. The average  $\rho$  between each structural property and each measure of site variability (entropy and evolutionary rates) can be seen in [Table 1](#) and [Table 2](#). The viral proteins experience much lower site variability. Figure 1 shows the correlations between CN and entropy for the virus and enzyme proteins. Even though on average the correlations in the enzyme proteins are larger as reported, by Yeh et al. (2014), the virus proteins still have correlation strengths that are comparable to some of the enzyme proteins with lower correlations.

The average site variability of a given protein does not seem to be a significant determinant of the strength of structural correlations (Figure 1A, 2A, 3A). However, when examining the variance of entropy there is a clear trend within the enzyme proteins. Proteins with a higher variance in site variability across the protein typically have lower correlations (Figure 1B, 2B, 3B). If you extrapolate the trend from the enzyme proteins, the viral proteins follow a similar trend. The correlation between the  $\rho$  between RSA and entropy and the variance of entropy is positive. The correlation is negative between local packing density (both WCN and CN) and the variance of entropy is negative. Unlike with entropy, there is no relationship between variance of evolutionary rates at sites and any of the measured structural properties. There also is a wide spread in the variance of the evolutionary rates across proteins for both the enzyme and viral proteins.

### 4 Discussion

SOME DISCUSSION.....

Need a section about: Rate4Site is completely different from the entropy variation and we do not know why. Explain the difference.

### 5 Acknowledgements

The authors acknowledge the Texas Advanced Computing Center (TACC) at The University of Texas at Austin for providing High-performance computing resources. ELJ is funded by a National Science Graduate Research Fellowship, grant number DGE-1110007. COW is funded by [Which grants??](#). AS is funded by [Which grants??](#). ....

### References

**Franzosa EA, Xia Y. 2009.** Structural determinants of protein evolution are context-sensitive at the residue level. *Mol. Biol. Evol.* **26**:2387–2395.

- Huang TT, Marcos ML, Hwang JK, Echave J. 2014.** A mechanistic stress model of protein evolution accounts for site-specific evolutionary rate and their relationship with packing and flexibility. *BMC Evol. Biol.* **14**:78.
- Kabsch W, Sander C. 1983.** Dictionary of protein secondary structure: pattern recognition of hydrogen-bonded and geometrical features. *Biopolymers* **22**:2577–2637.
- Katoh K, Kuma KI, Toh H, Miyata T. 2005.** Mafft version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Res.* **33**:511–518.
- Katoh K, Misawa K, Kuma K, Miyata T. 2002.** Mafft: a novel method for rapid multiple sequence alignment based on fast fourier transform. *Nucleic Acids Res.* **30**:3059–3066.
- Liao H, Yeh W, Chiang D, Jernigan RL, Lustig B. 2005.** Protein sequence entropy is closely related to packing density and hydrophobicity. *PEDS* **18**:59–64.
- Mayrose I, Graur D, Ben-Tal N, Pupko T. 2004.** Comparison of site-specific rate-inference methods for protein sequences; empirical bayesian methods are superior. *Mol. Biol. Evol.* **21**:1781–1791.
- Porter CT, Bartlett GJ, Thornton JM. 2004.** The catalytic site atlas: a resource of catalytic sites and residues identified in enzymes using structural data. *Nucleic Acids Res.* **32**:D129–D133.
- Ramsey DC, Scherrer MP, Zhou T, Wilke CO. 2011.** The relationship between relative solvent accessibility and evolutionary rate in protein evolution. *Genetics* **188**:479–488.
- Scherrer MP, Meyer AG, Wilke CO. 2012.** Modeling coding-sequence evolution within the context residue solvent accessibility. *BMC Evol. Biol.* **12**:179.
- Shahmoradi A, Sydykova DK, Spielman SJ, Jackson EL, Dawson ET, Meyer AG, Wilke CO. 2014.** Predicting evolutionary site variability from structure in viral proteins: Buriedness, packing, flexibility, and design. *J. Mol. Evol.* **79**:130–142.
- Stamatakis A. 2006.** Raxml-v1-hpc: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**:2688–2690.
- Stamatakis A. 2014.** Raxml version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**:1312–1313.
- Tien MZ, Meyer AG, Sydykova DK, Spielman SJ. 2013.** Maximum allowed solvent accessibilities of residues in proteins. *PLOS ONE* **8**:e80635.
- Wilke CO, Drummond DA. 2010.** Signatures of protein biophysics in coding sequence evolution. *Curr. Opin. Struct.* **20**:385–9.
- Yeh SW, Liu JW, Yu SH, Shih CH, Hwang JK, Echave J. 2014.** Site-specific structural constraints on protein sequence evolutionary divergence: Local packing density versus solvent exposure. *Mol. Biol. Evol.* **31**:135–139.

# Figures

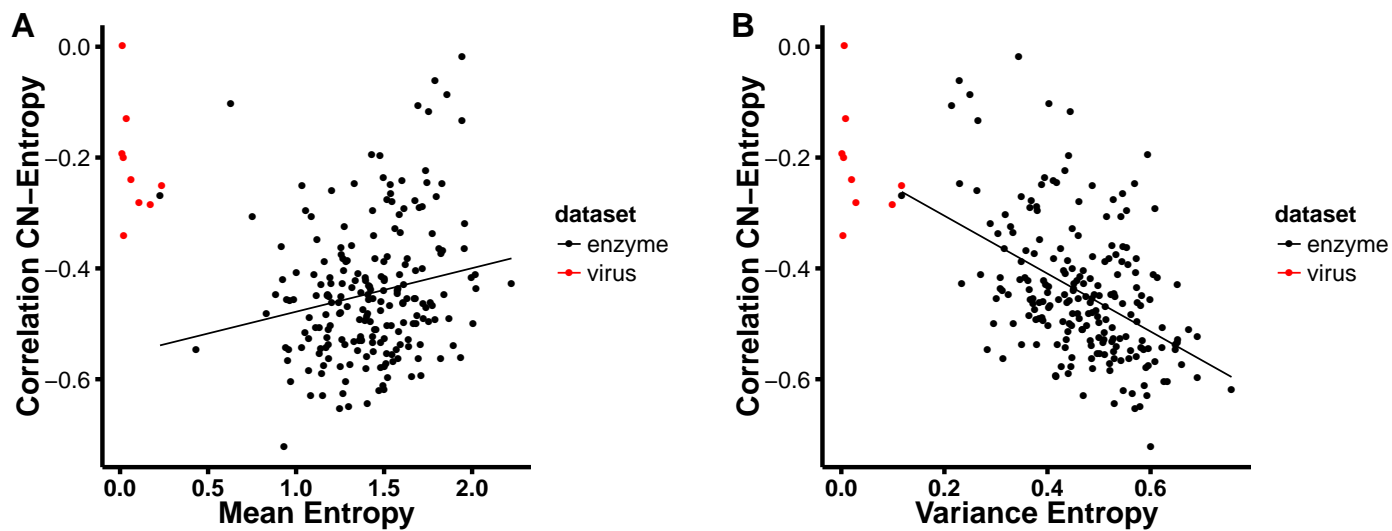


Figure 1: NEED A CAPTION!!!.

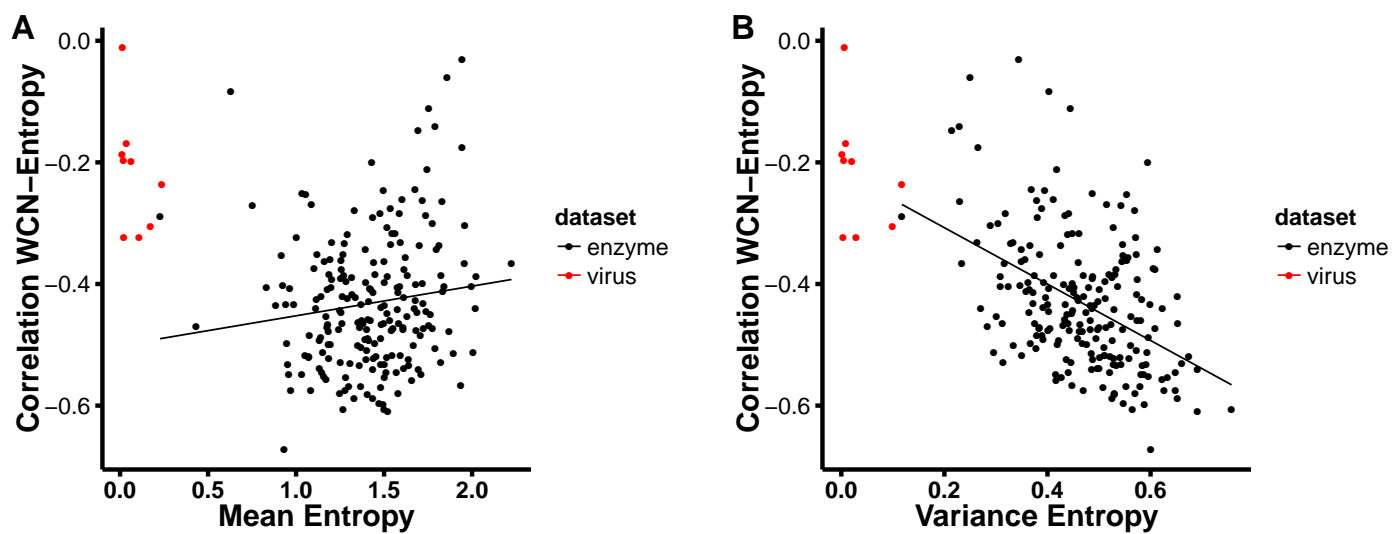


Figure 2: NEED A CAPTION!!!.

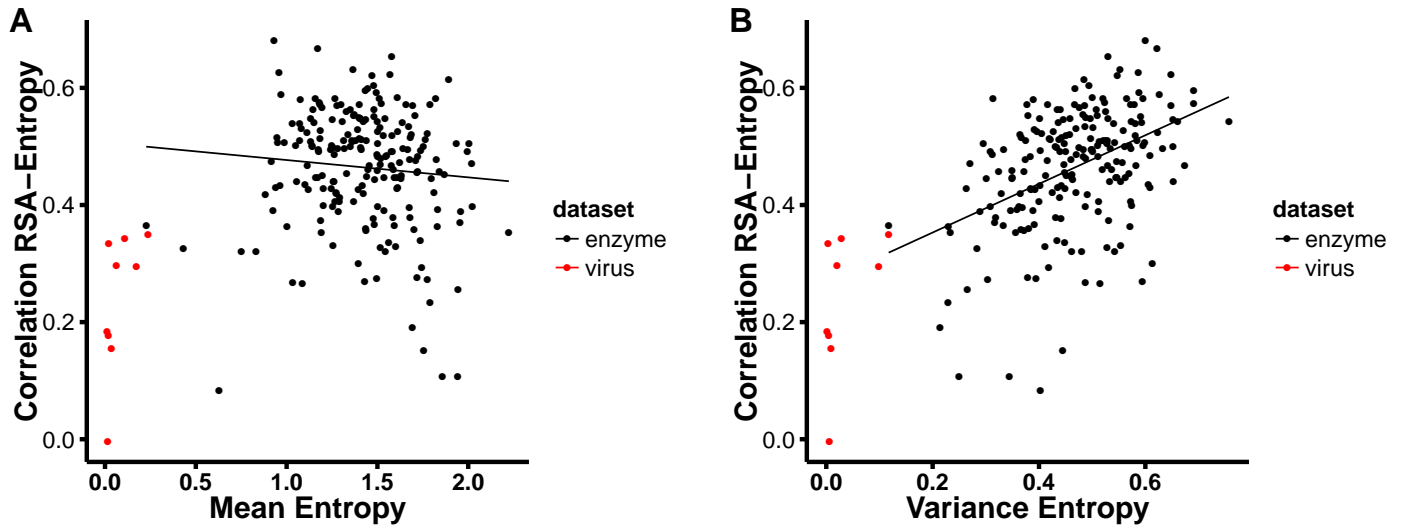


Figure 3: NEED A CAPTION!!!.

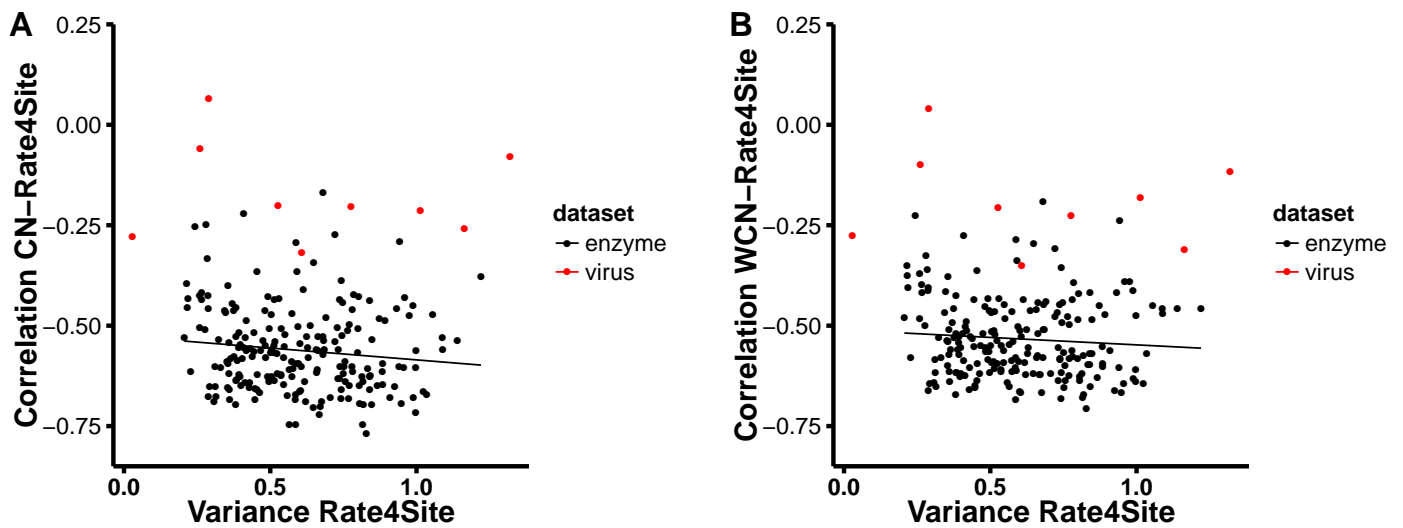


Figure 4: NEED A CAPTION!!!.

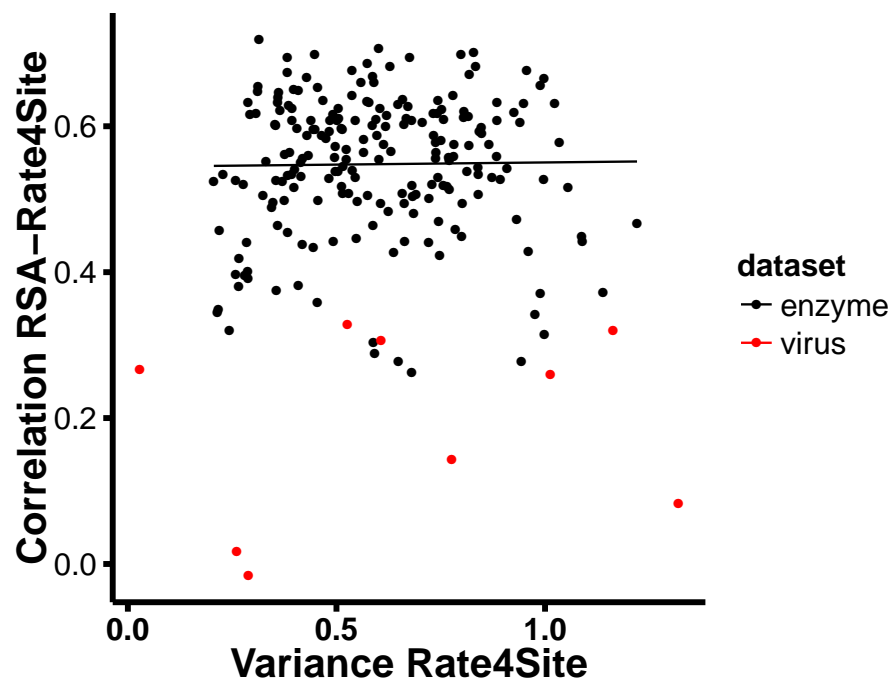


Figure 5: NEED A CAPTION!!!.

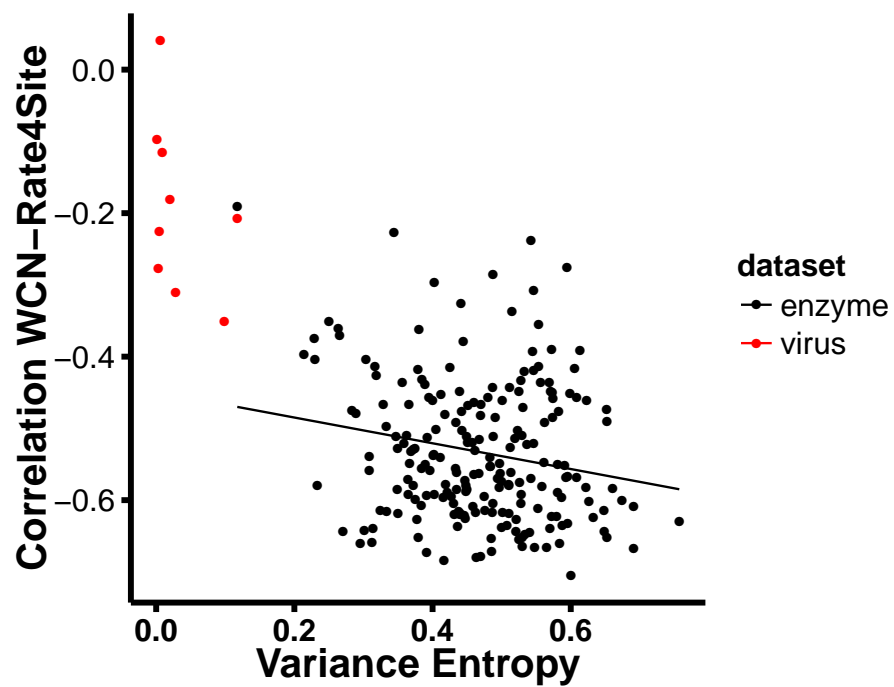


Figure 6: NEED A CAPTION!!!.