Applied Statistics

A statistical approach in protein stability prediction

Here I explore a method for rapid prediction of protein stability upon change of residue composition. A bayesian probabilistic knowledge-based state of the art multibody multinomial model, MuMu, was investigated as a mean of predicting protein structure stability of mutated proteins in real-time. By using a readily available method for predictions of protein side-chains it was possible to develop a high-throughput method for mutating proteins and determining their stability by MuMu. The probabilities given by the MuMu model was compared to a dataset of 458 experimental ΔΔG values originating from 14 proteins. I seek to increase the MuMu models correlation to experimental determined ΔΔG values by investigating the data with statistical analysis. The dataset was divided into a series of subsets based on the mutated amino acids physical properties. None of the methods, however, did not reveal a significant relationship between the probabilities and ΔΔG values.

Introduction

Previous analysis of the MuMu model has shown that there is a correlation between MuMu probabilities and ΔΔG values. Previous analysis was done on a single protein with 95 mutations. See figure 1.

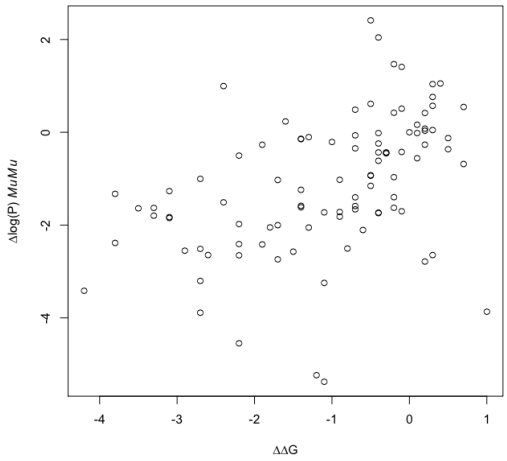


Figure 1. Data is not perfectly centered around a diagonal line. y-axis shows the change in lcogaritmich probability upon mutation, Δlog(P(A| X)). x-axis shows the change in change of free energy upon mutation.

A Pearson correlation analysis revealed a correlation of r = 0.45 and a hypothesis test rejected a null hypothesis of no correlation. r = 0.45 indicates a modest correlation (Taylor, R. 1990). However, the correlation is relatively high in terms of protein stability prediction. An increase in data size is naturally the next step to further asses the reliability of the MuMu model. The probability data was made by introducing mutations to known pdb structures of the proteins from where the ΔΔG values were obtained. The mutations was done by a program called SCWRL4. Next the probabilities of the wild type protein and all the mutated versions of the protein were determined by MuMu. All this was put together by a python script - allowing fast introduction of mutations and their influence on the probability. This influence was represented by a change in probability from wild type protein to mutated protein. A dataset of 95 Δlog(p) values.

In this project I rewrote the python script to allow for copy-pasting protein stability data, in ΔΔG, directly from:
"http:/www.abren.net/protherm/",

which is a thermodynamic database. From that I got 458 ΔΔG values. I generated 458 Δlog(p) values from the structure of the corresponding proteins. All this was stored as a pandas data frame so the data could be easily accessed and manipulated.

Analyzing the data

First I wanted to do a simple scatter plot as previously done. I wanted to make sure that my data was made correctly so I tried to replicate the data seen in figure 1. I ran the python script with the same protein, namely 1STN. The same plot was generated, not shown here. I then proceeded to generate the complete dataset from 16 proteins. The data was filtered of Null values and I ended up with 458 values of ΔΔG and 458 Δlog(p) values. I manually assessed the data to make sure that there were no errors.

I then proceeded to make the scatter plot, figure 2.

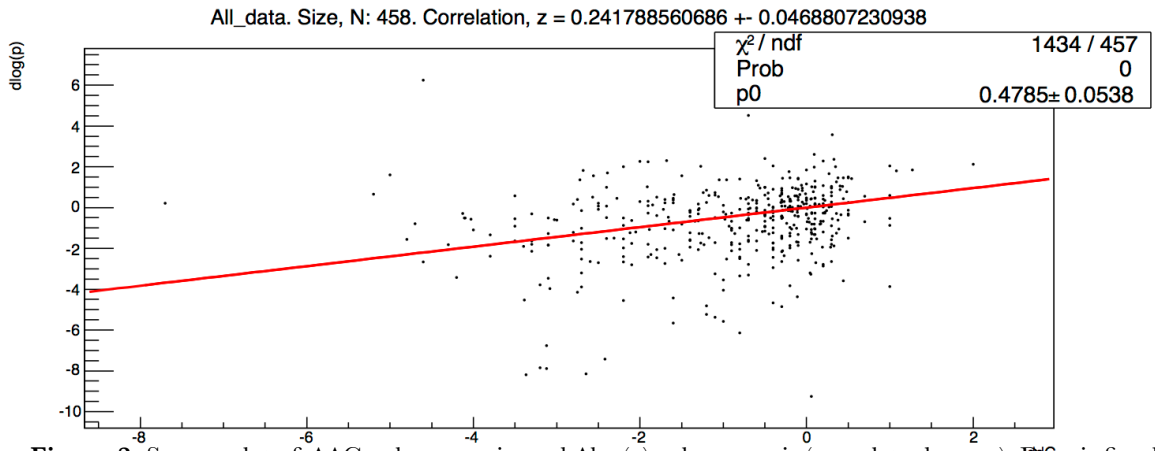


Figure 2. Scatter plot of ΔΔG values, x-axis, and Δlog(p) values, y-axis (complete data set). Data is fitted with a linear function.

The plot was fitted with a linear function. The assumption being that no change i probability would mean no change in stability and thus no change in ΔΔG. This means that the fit was without extra parameters. The first impression by looking at the plot is that there may be some, however little, correlation in between ΔΔG and Δlog(p). The Δlog(p) was calculated so that a negative value would indicate increasing stability which is the same for ΔΔG values. The chi-square of the fit is 1 434 and ndf is 457. This does not indicate a good fit and when we look at the probability that is extremely low we may conclude that it is indeed a bad fit.

I wanted to test a null hypothesis that there is no fit against an alternative hypothesis that there is a good fit. To do this I made a runs test. This revealed and extremely high sigma of 10.68 and a the run test gave a value of 4.48 sigma. This means that we are more than 4 sigma away. The alternative hypothesis can therefore be rejected.
The correlation, rho, was calculated using ROOT. The correlation coefficient can be transformed into the variable z, as the sample size is not *very large:*

$$z = \frac{1}{2}\ln\left(\frac{1+\hat{\rho}}{1-\hat{\rho}}\right).$$

z has a more gaussian shape than rho and the standard derivation of z is:

$$std = \frac{1}{\sqrt{N-3}}$$

This was calculated by ROOT and attached to all figures provided, see figure 2. The correlation coefficient of 0.25 is very low.

Different amino acids have different properties. It may be that the MuMu model is better at predicting changes in stability when the mutation is of a certain type. The dataset was split up into different categories to investigate this. First I choose to look at the most common classification of amino acids. I generated a python script that could filter the data based on different criteria. Figures are shown in are found in appendix, figure 1. Correlations from the fits are shown in table 1.

	z	error
All data	0,24	0,05
Mutated to charged amino acid (figure app. 1B)	0,44	0,11
Mutated to uncharged amino acid (figure app. 1C)	0,20	0,05
Mutated to aromatic amino acid (figure app. 1D)	-0,2	0,24
Uncharged amino acid mutated to charged amino acid (figure app. 1E)	0,25	0,08
Charged amino acid mutated to uncharged amino acid (figure app. 1F)	0,65	0,15
Mutated to hydrophobic amino acid (figure app. 1G)	0,22	0,06
Mutated to hydrophilic amino acid (figure app. 1H)	0,15	0,1
Hydrophobic amino acid mutated to hydrophilic amino acid (figure app. 1I)	0,43	0,17
Hydrophilic amino acid mutated to hydrophobic amino acid (figure app. 1J)	0,4	0,12

Table 1. Correlation coefficients, z and their errors. Based on subsets of the complete dataset from appendix, figure 1.

The plot with charged amino acid to uncharged (peptide) amino acid and the plot with the charged amino acid have relatively high correlation (speaking in terms of structure prediction), 0.65 +- 0.14 and 0.44 +- 0.11 (table 1 & appendix figure 1). It would seem that they have a high contribution to the correlation of the data. A manual inspection of the data reveals that the data does seem somewhat random it is not clear if that is the case without collecting more data. The correlation coefficient for changes of polar amino acids to hydrophobic amino acids is 0.39 +- 0.12. It does look like there is a linear tendency. MuMu may be better at predicting stability when there are mutations from hydrophilic to hydrophobic. It seems that mutations that lead to aromatic amino acids has an negative overall influence on the correlation. However it is inconclusive if this is the case due to the low sample size of 19.

The MuMu model is trained on non-membrane proteins. All proteins chosen here are therefore non-membrane. It may be that MuMu is more sensitive to different

kind of proteins and that it is better at predicting the stability of some of the chosen proteins than others. This time I split up the data into subcategories that are the proteins. Same analysis of correlation was done. See appendix, protein figures. The correlation coefficients can be seen in table 2. There is negative influence on the correlation coefficient from a few of the protein, especially 1VQB, 1CYO. 3SSI have a perfect correlation however the plot shows that it is caused by a very bad fit. 1STN has about the same correlation as seen in the previous study. 1PGA and 1BVC has a high correlation but the standard derivation is also quite high thus making it unreliable. 1ROP has the highest correlation even with its error of 0.25. The sample size of 1ROP is not large enough to say anything conclusive.

	z	error
All data	0,24	0,05
1BNI	0,23	0,08
1BPI	0,03	0,25
1BVC	0,63	0,41
1C90	0,28	0,29
1CSP	0,17	0,25
1CYO	-0,32	0,41
1FTG	-0,1	0,41
1IGV	-	-
1PGA	0,64	0,41
1ROP	0,75	0,25
1STN	0,47	0,11
1VQB	-0,2	0,25
2LZM	0,29	0,12
2RN2	0,16	0,32
3SSI	1	0,45

Conclusion

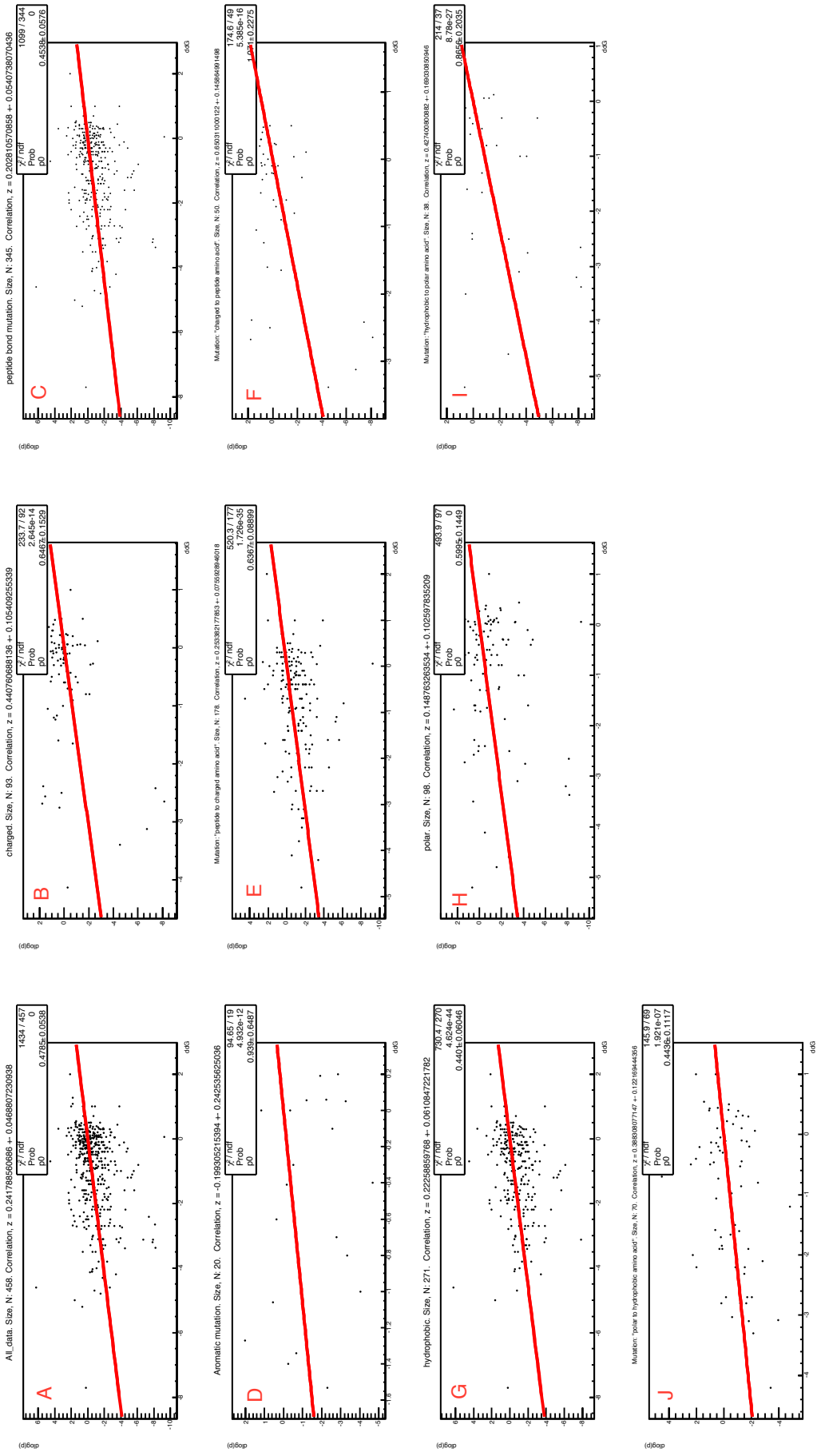
A recurring thing is the need for a larger sample size. This is a problem especially when looking at the subsets. There is an indication that MuMu might be better at predicting stability of proteins with mutations of hydrophobic amino acids to hydrophilic. The fit for the entire dataset is not good. The predictions are not consistent enough to use it as a way of determine ΔΔG values on the basis of MuMu values. That is with the current dataset.

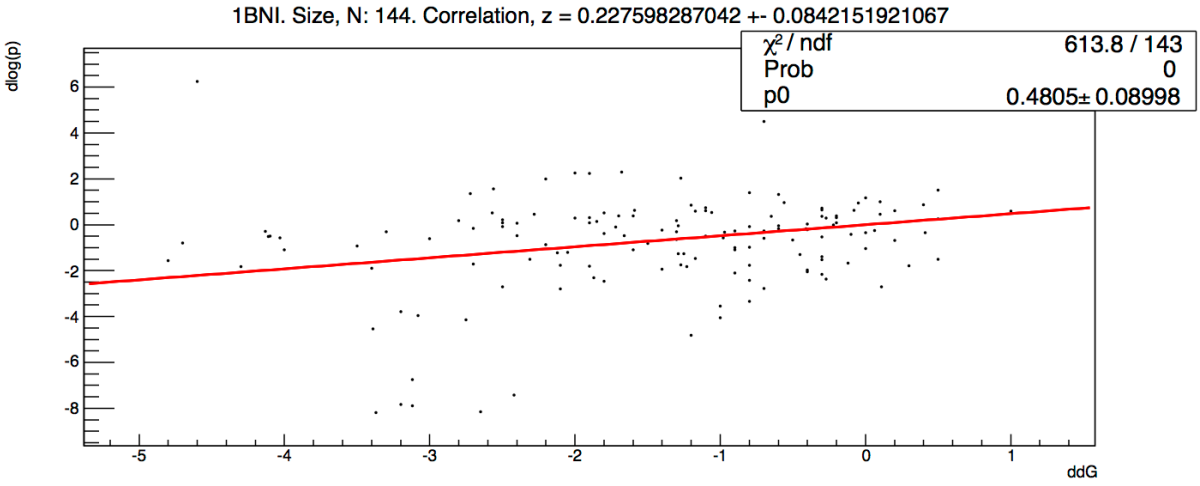
Literature

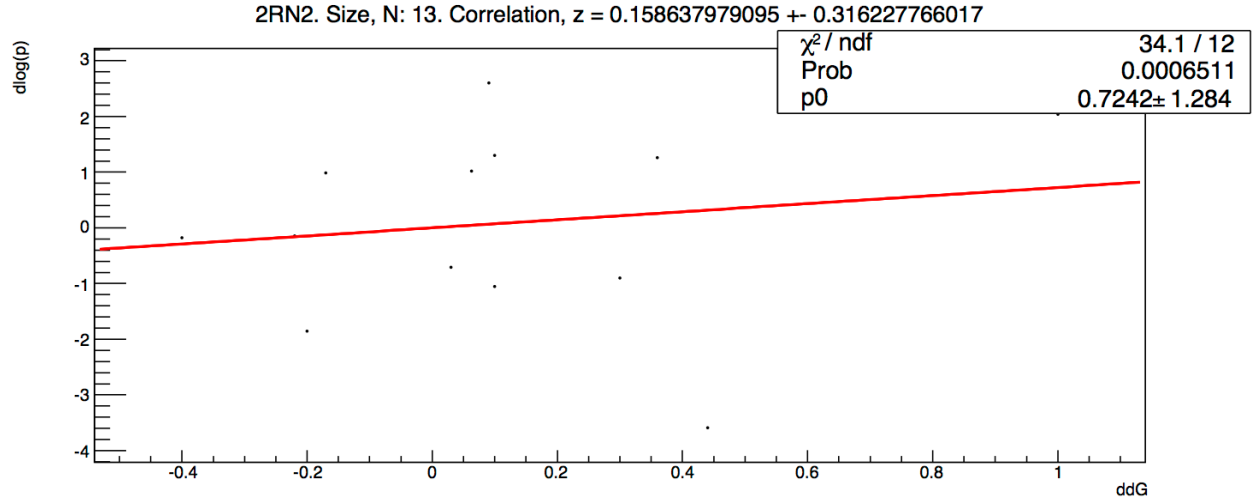
Taylor, R. (1990). Interpretation of the Correlation Coefficient: A Basic Review. Journal of Diagnostic Medical Sonography, 6(1), 35–39. doi: 10.1177/875647939000600106

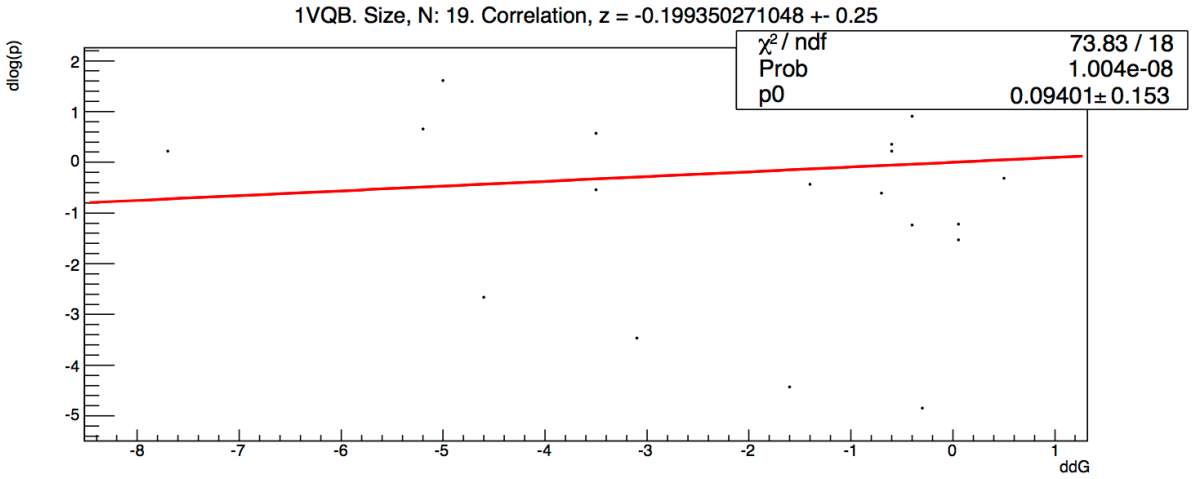
Roger J. Barlow. Statistics: A Guide to the Use of Statistical Methods in the Physical Sciences.

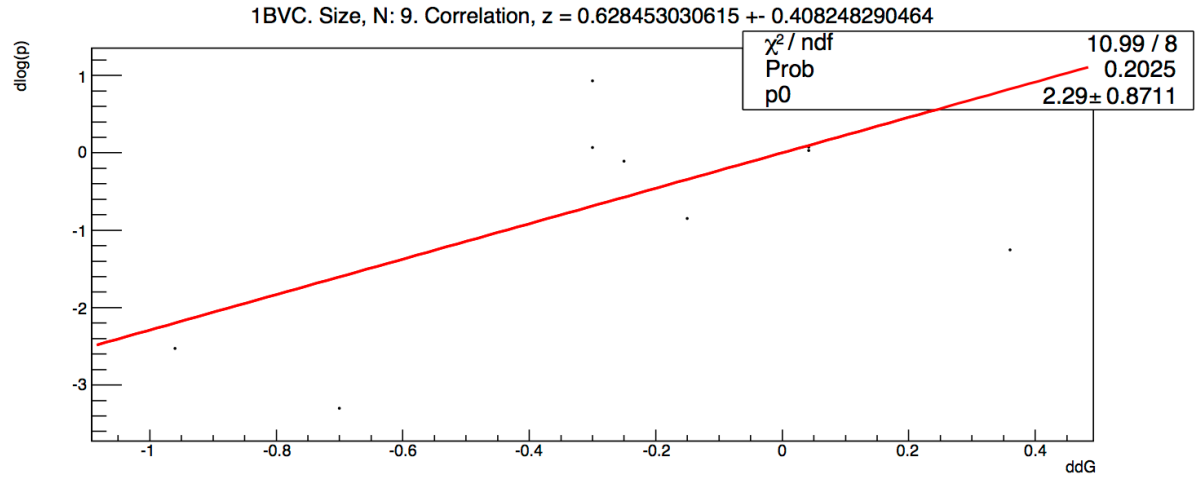
Appendix

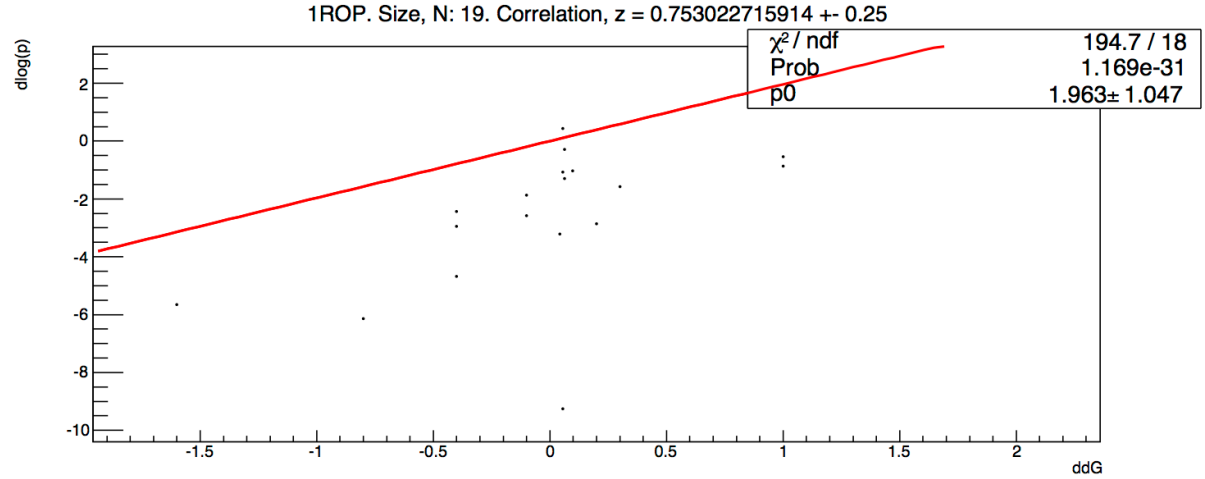

All_data. Size, N: 458. Correlation, z = 0.24178560686 + 0.0468807230938
χ² / ndf 1434 / 457
Prob 0
p0 0.4785± 0.0538
A
Aromatic mutation. Size, N: 20. Correlation, z = -0.199305215394 + 0.249253625036
χ² / ndf 94.65 / 19
Prob 4.932e-12
p0 0.939± 0.6487
D
hydrophobic. Size, N: 271. Correlation, z = 0.222588559768 + 0.0610847221782
χ² / ndf 790.4 / 270
Prob 4.624e-44
p0 0.4401± 0.06046
G
Mutation: "polar to hydrophobic amino acid". Size, N: 70. Correlation, z = 0.388080771471 + 0.0121619444356
χ² / ndf 145.9 / 69
Prob 1.921e-07
p0 0.4439± 0.1117
J
peptide bond mutation. Size, N: 345. Correlation, z = 0.202810570858 + 0.0540738070436
χ² / ndf 1099 / 344
Prob 0
p0 0.4538± 0.0576
C
Mutation: "charged to peptide amino acid". Size, N: 50. Correlation, z = 0.693031000122 + 0.143864991498
χ² / ndf 174.6 / 49
Prob 5.385e-16
p0 1.038± 0.2275
F
Mutation: "hydrophobic to polar amino acid". Size, N: 38. Correlation, z = 0.427400800982 + 0.0180909859346
χ² / ndf 214 / 37
Prob 8.79e-27
p0 0.865± 0.2035
I
charged. Size, N: 93. Correlation, z = 0.440760688136 + 0.105409255339
χ² / ndf 233.7 / 92
Prob 2.645e-14
p0 0.6467± 0.1529
B
Mutation: "peptide to charged amino acid". Size, N: 178. Correlation, z = 0.253821177853 + 0.0759289840018
χ² / ndf 520.3 / 177
Prob 1.726e-35
p0 0.6367± 0.08899
E
polar. Size, N: 98. Correlation, z = 0.148763263534 + 0.102597835209
χ² / ndf 493.9 / 97
Prob 0
p0 0.5995± 0.1449
H
(d)δαρ
ddδ

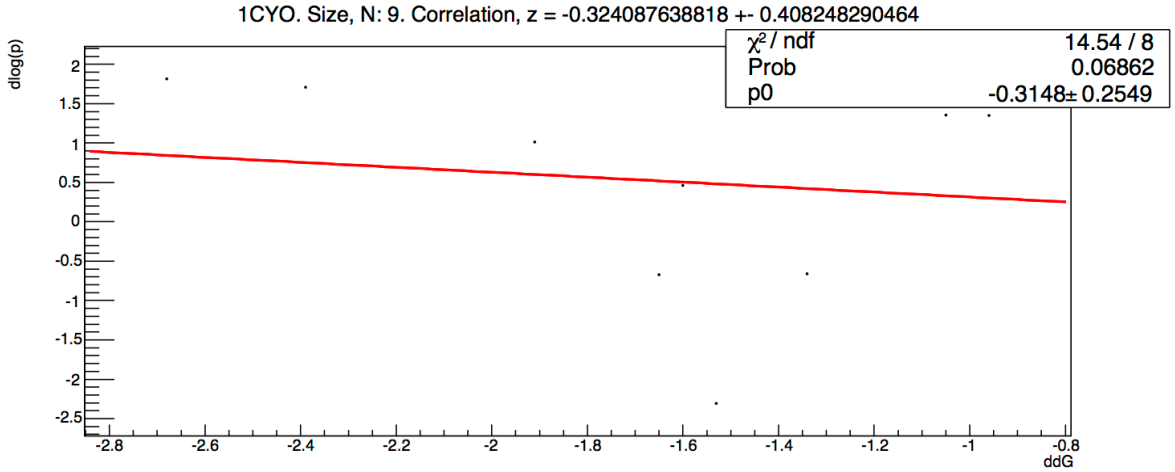

1BNI. Size, N: 144. Correlation, z = 0.227598287042 +- 0.0842151921067
χ² / ndf 613.8 / 143
Prob 0
p0 0.4805± 0.08998
dlog(p)
ddG

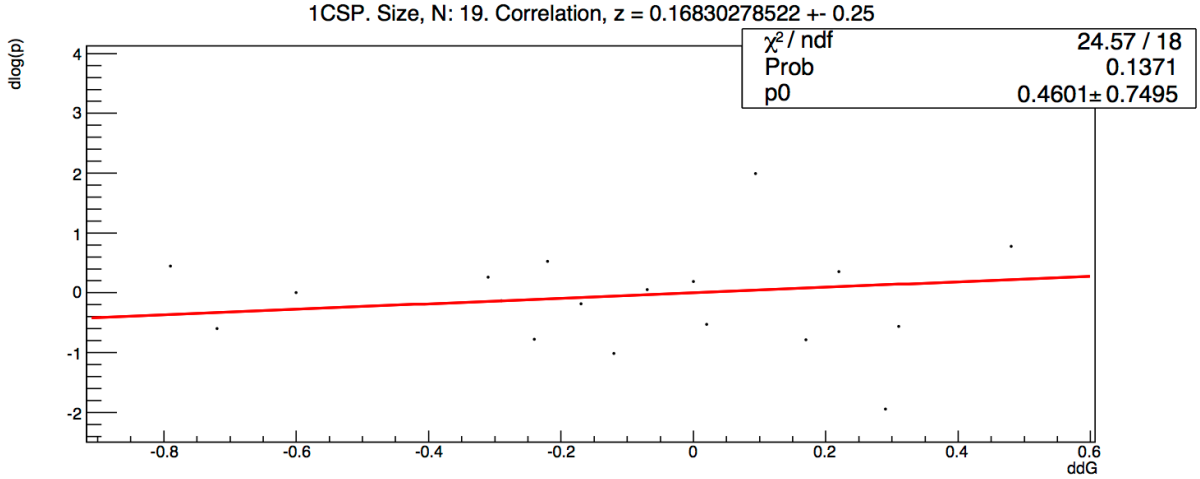

2RN2. Size, N: 13. Correlation, z = 0.158637979095 +- 0.316227766017
χ² / ndf 34.1 / 12
Prob 0.0006511
p0 0.7242± 1.284
dlog(p)
ddG

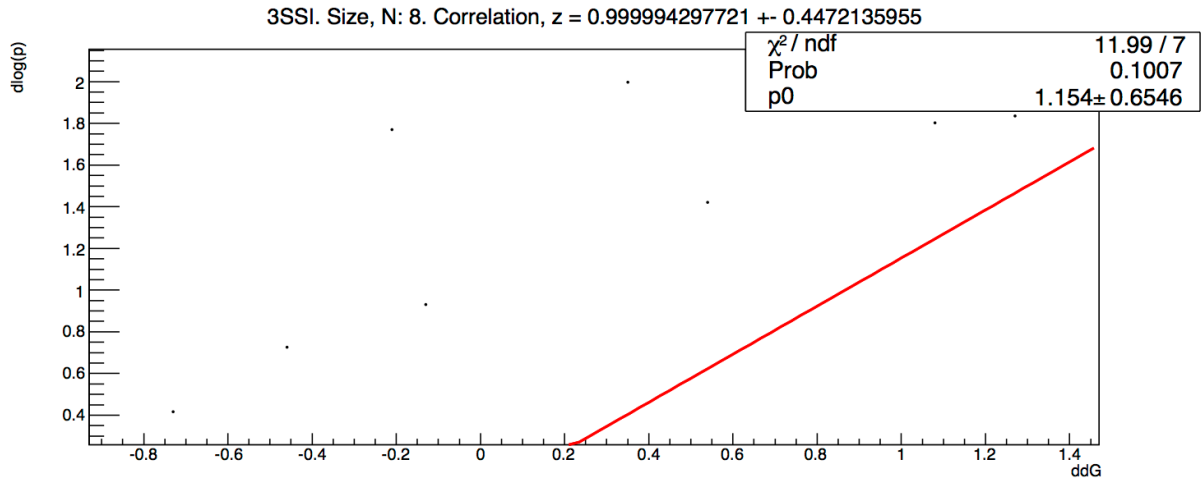

1VQB. Size, N: 19. Correlation, z = -0.199350271048 +- 0.25
χ² / ndf 73.83 / 18
Prob 1.004e-08
p0 0.09401± 0.153
dlog(p)
ddG

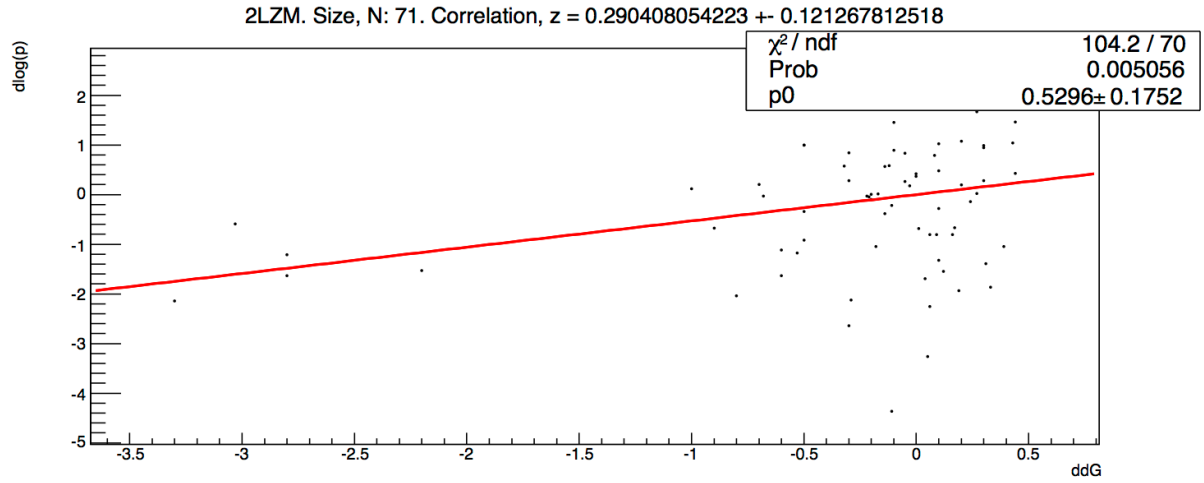

1BVC. Size, N: 9. Correlation, z = 0.628453030615 +- 0.408248290464
χ² / ndf 10.99 / 8
Prob 0.2025
p0 2.29± 0.8711
dlog(p)
ddG

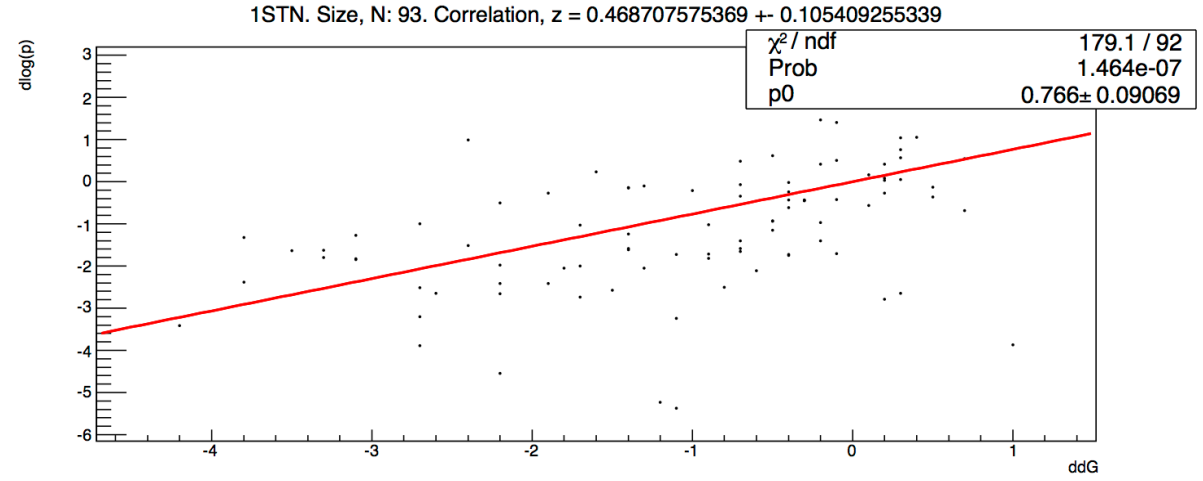

1ROP. Size, N: 19. Correlation, z = 0.753022715914 +- 0.25
χ² / ndf 194.7 / 18
Prob 1.169e-31
p0 1.963± 1.047
dlog(p)
ddG

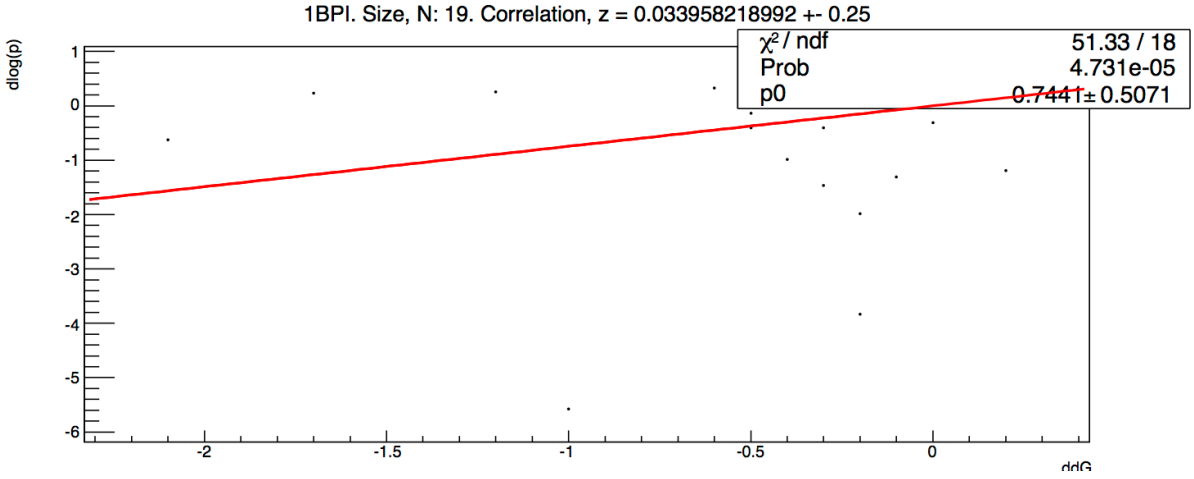

1CYO. Size, N: 9. Correlation, z = -0.324087638818 +- 0.408248290464
χ² / ndf 14.54 / 8
Prob 0.06862
p0 -0.3148± 0.2549
dlog(p)
ddG

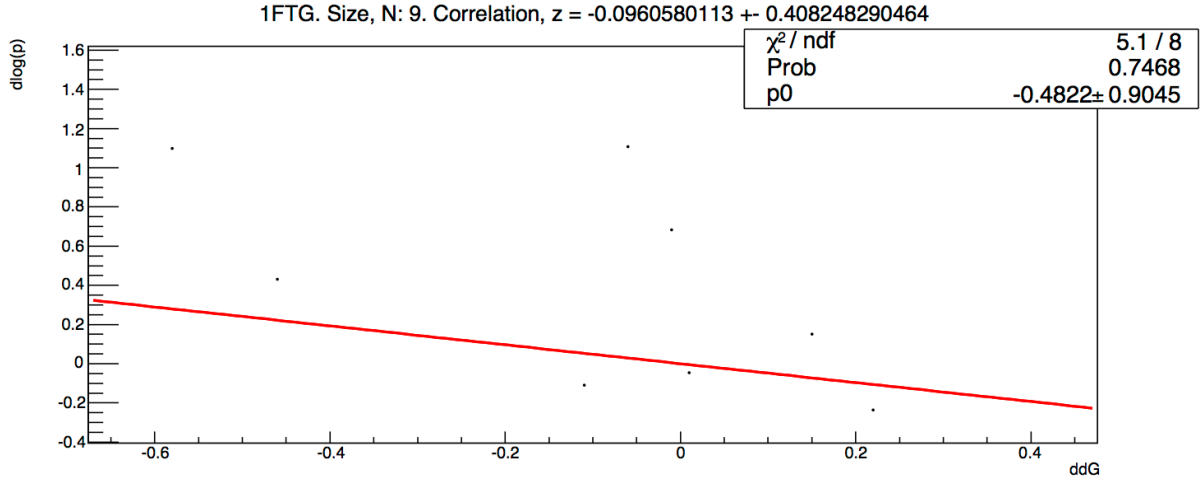

1CSP. Size, N: 19. Correlation, z = 0.16830278522 +- 0.25
χ² / ndf 24.57 / 18
Prob 0.1371
p0 0.4601± 0.7495
dlog(p)
ddG

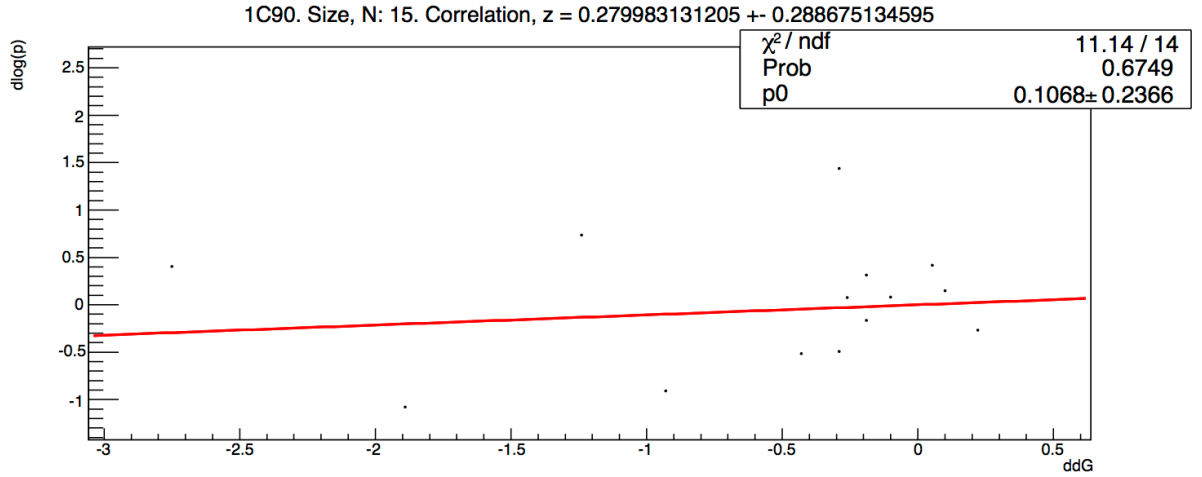

3SSI. Size, N: 8. Correlation, z = 0.999994297721 +- 0.4472135955
χ² / ndf 11.99 / 7
Prob 0.1007
p0 1.154± 0.6546
dlog(p)
ddG

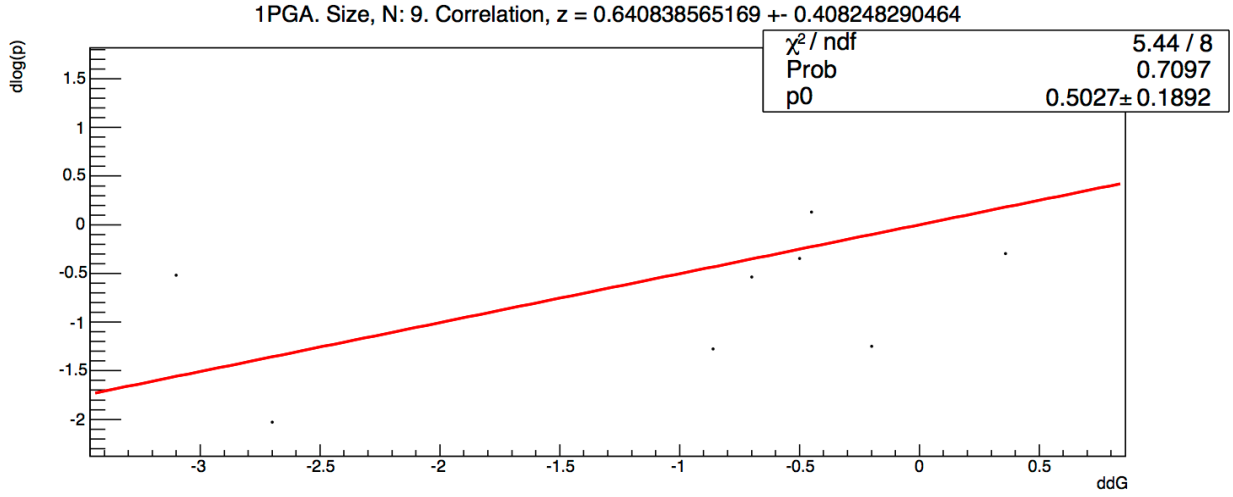

2LZM. Size, N: 71. Correlation, z = 0.290408054223 +- 0.121267812518
χ² / ndf 104.2 / 70
Prob 0.005056
p0 0.5296± 0.1752
dlog(p)
ddG


1STN. Size, N: 93. Correlation, z = 0.468707575369 +- 0.105409255339
χ² / ndf 179.1 / 92
Prob 1.464e-07
p0 0.766± 0.09069
dlog(p)
ddG


1BPI. Size, N: 19. Correlation, z = 0.033958218992 +- 0.25
χ² / ndf 51.33 / 18
Prob 4.731e-05
p0 0.7441± 0.5071
dlog(p)
ddG


1FTG. Size, N: 9. Correlation, z = -0.0960580113 +- 0.408248290464
χ² / ndf 5.1 / 8
Prob 0.7468
p0 -0.4822± 0.9045
dlog(p)
ddG


1C90. Size, N: 15. Correlation, z = 0.279983131205 +- 0.288675134595
χ² / ndf 11.14 / 14
Prob 0.6749
p0 0.1068± 0.2366
dlog(p)
ddG


1PGA. Size, N: 9. Correlation, z = 0.640838565169 +- 0.408248290464
χ² / ndf 5.44 / 8
Prob 0.7097
p0 0.5027± 0.1892
dlog(p)
ddG