

Charles University
Faculty of Science

Study programme: Bioinformatics

Branch of study: Bioinformatics



Kateřina Břicháčková

Use of residue-level annotations for structural prediction of
protein-ligand binding sites

Využití anotací primární struktury pro strukturní predikci
protein-ligand aktivních míst

Master thesis

Supervisor: RNDr. David Hoksza, Ph.D.

Prague, 2020

Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, XXX

Kateřina Břicháčková

Poděkování

Ondrovi :P

Acknowledgement

Ondrovi :P

Abstract

abstract

Keywords: keyword1, keyword2

Abstrakt

abstract

Klíčová slova: keyword1, keyword2

Contents

1	Introduction	2
1.1	Section1	2
2	Method	3
2.1	Hypothesis testing	3
2.1.1	Welch’s test	4
2.1.2	Fisher’s exact test	5
2.1.3	Chi-squared test	5
2.2	Data	5
2.3	Implementation in Python	5
	Conclusion	6
	List of Abbreviations	7
	Bibliography	8
A	Attachments	10
A.1	attachment1	10
A.2	attachment2	10

1. Introduction

1.1 Section1

2. Method

To find properties mapped on the protein primary structure which are possibly important for prediction of protein-ligand binding sites, statistical analysis will have the crucial role. It is a great way to explore the big amounts of accessible data and it can potentially help to discover underlying patterns and draw inferences from the data.

This chapter describes the method that was used to analyse the *statistical significance* of the properties and to distinguish the ones that stand out in the known protein-ligand binding sites.

2.1 Hypothesis testing

Hypothesis testing is a method of statistical inference. Its goal is to infer properties of a *statistical population*, i.e. a set of similar items or events. In this work, two populations will be compared: we take values of a property for all the amino acids across all the proteins in the dataset and then compare the ones in binding sites and outside of binding sites.

A dataset usually contains a subset sampled from a larger population, rather than the whole population. This subset is called a *statistical sample*. It should represent the population well and be unbiased.

A *hypothesis* makes a statement about an unknown population parameter. In a hypothesis testing problem, an experimenter states two complementary hypotheses: the *null hypothesis* and the *alternative hypothesis*, denoted by H_0 and H_1 , respectively. The null hypothesis comprises a subset of possible parameters and the alternative hypothesis comprises the supplement, so that all the possible parameters are covered.

In a hypothesis testing problem, an experimenter should come to one of the conclusions: to either accept H_0 , or to reject H_0 and accept H_1 .

To decide which one of two complementary hypotheses is true, an experimenter employs a suitable *hypothesis test*. A hypothesis test is a rule that specifies for which sample values the H_0 is accepted as true and for which sample values it is rejected, and therefore H_1 is accepted as true. A hypothesis test is usually specified in terms of a test statistic (i.e. a function of the sample) [1].

As one may expect, the tests are not error-proof and a mistake can be made in the decision of whether to accept or reject the null hypothesis. There are two types of errors in hypothesis testing, commonly known as *Type I error* and *Type II error*. The test has made a Type I error if it incorrectly rejects a true null hypothesis. If, on the other hand, a null hypothesis is accepted and it is not true, a Type II error has been made. Both situations are depicted in the Table 2.1. The ideal test would have both error probabilities equal to zero. Nevertheless, in most cases it is not possible to make both error probabilities arbitrarily small for a fixed sample size [1].

To control statistical significance of the result, a study defines a threshold called *significance level*, a constant denoted by α . It represents the probability of making a Type I error, in other words, the probability that the study rejects the null hypothesis when it is true.

One way to report the result of the test would be simply to tell whether the null hypothesis was accepted or rejected at the given significance level. However, most researchers choose to report a certain kind of test statistic (function of a sample X), the so-called *p-value*. Smaller values of $p(X)$ give stronger evidence for rejecting the null hypothesis. The null hypothesis is rejected when $p(X) \leq \alpha$. Hence, we are able to determine the smallest significance level at which the hypothesis would be accepted/re-

		Prediction	
		Accept H_0	Reject H_0
Truth	H_0	Correct (true positive)	Type I error (false positive)
	H_1	Type II error (false negative)	Correct (true negative)

Table 2.1: Type I and II Error in hypothesis testing.

jected. P-value gives an idea of how strongly the data contradict the null hypothesis; furthermore, it allows other researchers to make a decision according to the significance level of their choice [1, 3, 9].

It is suggested that the significance level for a study is set prior to any data collection [7]. The typical choices in practice are $\alpha = 0.01, 0.05$ or 0.10 [1].

One should be aware that by fixing the significance level of the test, the experimenter is controlling only the Type I error probabilities. The probability of the Type II error is subject to factors such as the accuracy and completeness of the data [9].

Let's suppose an experimenter has a research hypothesis that he or she hopes to prove, but does not want to risk accepting it without convincing data support. In this case, the test should be set up in such a way that the research hypothesis corresponds to the alternative hypothesis, not the null hypothesis. By specifying a small significance level α , the experimenter thus controls the probability of the Type I error. In other words, the probability of accepting the research hypothesis when it is not true would be α at most [1].

2.1.1 Welch's test

Welch's unequal variances t-test, or Welch's test in short, is a two-sample hypothesis test used to decide whether two populations have different central tendencies (means or medians). The decision is made based on the samples from the two populations. It is a more robust alteration of the widely used Student's t-test [10].

Both Student's and Welch's t-test assume that the two examined populations follow a normal distribution [10]. Nevertheless, when testing for the equality of means of "large enough samples", the normality assumption can be violated thanks to the large sample theory and the Central Limit Theorem [3]. It has been shown in previous studies that for large samples, the statistical significance level is protected not only for normally distributed data, but also for many non-normal distributions; moreover, in case of Welch's test, this is true even for unequal variances [5, 13, 14]. According to Lehmann and Romano [3], the Type II error is also relatively insensitive to non-normality. Many articles and textbooks mention that when the sample sizes are small, nonparametric tests (i.e. tests that do not assume a specific distribution) such as the Mann-Whitney test [6] should be considered as an alternative to t-tests. However, t-tests become superior when sample sizes increase [5, 11]. The simulations made by Lumley *et al.* [5] show that "sufficiently large sample size" means under 100 in most cases. Even for extremely non-normal data, the sufficient size is at most 500. This suggests that the choice of Welch's test is legitimate for this work.

The problem of the Student's t-test is that it performs badly when the variances of the two compared populations are unequal. Both Type I and Type II errors are nega-

tively affected by violation of the equal variances assumption. The unequal variances can be less problematic if sample sizes are similar, but in practice, that is not always the case [8].

Unlike Student's t-test, Welch's test does not assume equal variances of the populations. It performs well when the samples have unequal variances; furthermore, it can be used even when the samples have unequal sizes [2].

Some researchers tend to pre-test for variance equality by a preliminary test of variances (such as Levene's [4], Bartlett's [?] or Brown-Forsythe test [?]) and then choose whether to use Student's or Welch's t-test. However, although this approach persists in some textbooks and software packages, it is not recommended by statisticians. As a preliminary test itself is subject to Type I and II errors, this two-stage procedure would not protect the significance level and could lead to incorrect decisions. One should be aware of the fact that even if the test suggested that the samples variances are nearly equal, it would not mean that the whole population variances could not differ to a larger extent [12]. Some researchers may try to make the significance level of a preliminary test more strict, so that they could be more confident about the choice of the subsequent test; however, as the significance level decreases, the performance of the compound test paradoxically gets worse. According to Zimmerman [12], "a higher Type I error rate of the preliminary test actually improves the performance of the compound test". This suggests that using the preliminary test is not correct in principle.

Welch's test should be used whenever the researcher is not sure that the variances are truly equal. Ruxton [8] even suggests the routine use of Welch's test. When the sample sizes and variances are equal, both tests perform similarly. When dealing with unequal variances and unequal sample sizes, Welch's test is more robust than Student's t-test and the Type I error rate does not deviate far from the nominal value [2]. Hence, Welch's test can be applied without any significant disadvantages to Student's t-test.

For all the reasons stated above, Welch's test seems to be the best choice for the purpose of this study. It has the best combination of performance and ease of use, the calculation is straightforward and it is available in commonly used statistics packages.

2.1.2 Fisher's exact test

2.1.3 Chi-squared test

2.2 Data

plus significance level, null hypothesis, alternative hypothesis

2.3 Implementation in Python

Conclusion

Conclusion.

List of Abbreviations

AA Amino acid
atd a tak dale

Bibliography

- [1] George Casella and Roger L Berger. *Statistical inference*, volume 2. Duxbury Pacific Grove, CA, 2002.
- [2] B. Derrick and P. White. Why welch's test is type i error robust. *The Quantitative Methods for Psychology*, 12(1):30–38, jan 2016. doi: 10.20982/tqmp.12.1.p030.
- [3] Joseph P. Romano Erich L. Lehmann. *Testing Statistical Hypotheses*. Springer New York, 2008. ISBN 0387988645. URL https://www.ebook.de/de/product/3186913/erich_l_lehmann_joseph_p_romano_testing_statistical_hypotheses.html.
- [4] Howard Levene. Robust tests for equality of variances. In Ingram Olkin, editor, *Contributions to Probability and Statistics: Essays in Honor of Harold Hotelling*, pages 278–292. Stanford University Press.
- [5] Thomas Lumley, Paula Diehr, Scott Emerson, and Lu Chen. The importance of the normality assumption in large public health data sets. *Annual Review of Public Health*, 23(1):151–169, may 2002. doi: 10.1146/annurev.publhealth.23.100901.140546.
- [6] H. B. Mann and D. R. Whitney. On a test of whether one of two random variables is stochastically larger than the other. *The Annals of Mathematical Statistics*, 18(1):50–60, mar 1947. doi: 10.1214/aoms/1177730491.
- [7] Jerzy Neyman and Egon S Pearson. *The testing of statistical hypotheses in relation to probabilities a priori*, volume 29. 1933.
- [8] Graeme D. Ruxton. The unequal variance t-test is an underused alternative to student's t-test and the mann-whitney u test. *Behavioral Ecology*, 17(4):688–690, may 2006. doi: 10.1093/beheco/ark016.
- [9] Pak C. Sham and Shaun M. Purcell. Statistical power and significance testing in large-scale genetic studies. *Nature Reviews Genetics*, 15(5):335–346, apr 2014. doi: 10.1038/nrg3706.
- [10] B. L. WELCH. THE GENERALIZATION OF 'STUDENT'S' PROBLEM WHEN SEVERAL DIFFERENT POPULATION VARLANCES ARE INVOLVED. *Biometrika*, 34(1-2):28–35, 1947. doi: 10.1093/biomet/34.1-2.28.
- [11] Donald W. Zimmerman. Invalidation of parametric and nonparametric statistical tests by concurrent violation of two assumptions. *The Journal of Experimental Education*, 67(1):55–68, jan 1998. doi: 10.1080/00220979809598344.
- [12] Donald W. Zimmerman. A note on preliminary tests of equality of variances. *British Journal of Mathematical and Statistical Psychology*, 57(1):173–181, may 2004. doi: 10.1348/000711004849222.
- [13] Donald W. Zimmerman and Bruno D. Zumbo. Rank transformations and the power of the student t test and welch t' test for non-normal populations with unequal variances. *Canadian Journal of Experimental Psychology/Revue canadienne de psychologie expérimentale*, 47(3):523–539, 1993. doi: 10.1037/h0078850.

- [14] Bruno D. Zumbo and Daniel Coulombe. Investigation of the robust rank-order test for non-normal populations with unequal variances: The case of reaction time. *Canadian Journal of Experimental Psychology/Revue canadienne de psychologie expérimentale*, 51(2):139–150, 1997. doi: 10.1037/1196-1961.51.2.139.

A. Attachments

The attached CD contains two attachments:

A.1 attachment1

blabla

A.2 attachment2

blabla