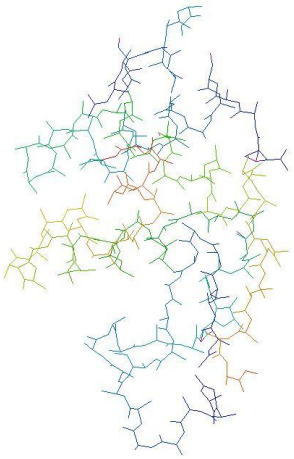
DESY SUMMER STUDENT PROGRAM 2009 HAMBURG



REPORT OF PHASING MACROMOLECULAR STRUCTURES WITH UV-INDUCED STRUCTURAL CHANGES

DAWID DZIADOŃ

JAGIELLONIAN UNIVERSITY

FACULTY OF MATHEMATICAL AND NATURAL SCIENCES

SUPERVISOR: SANTOSH PANJIKAR, PH.D.

Introduction

The only technique that allows direct visualization of macromolecular structure at the atomic level is X-ray diffraction analysis. This technique is used to study biological molecules such as proteins, viruses and nucleic acids.

In order to calculate electron densities from a diffraction experiment, solve the structure, it is needed to determine three important information in an analysis. The indices of a reflection (h,k,l), the intensity of the reflections, I(hkl), and the phase angles of the reflections, α(hkl) are essential to resolve macromolecular structure. Only indices of a reflection and phase of a reflection can be determined directly by the experiment.

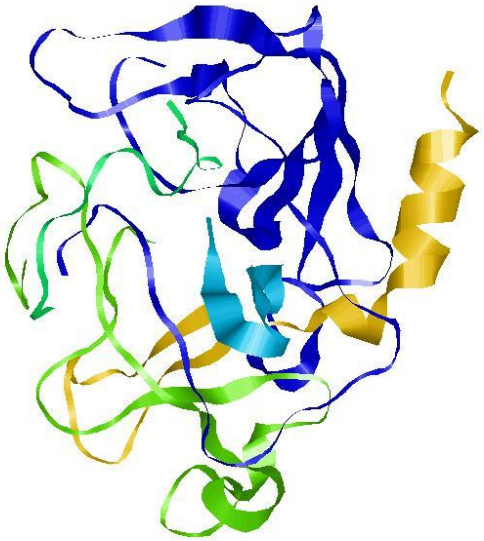


FIGURE 1 RESOLVED STRUCTURE OF ELASTASE

Nowadays there is a numerous investigations for a macromolecular structures but no mathematical procedure exists to solve phase problem from the beging for crystals of macromolecules.

Radiation damage induced phasing

To solve macromolecular structures, the radiation-damage-induced phasing (RIP) method can be used. In experiment specific damages was caused with UV-light in a different time of exposure. The method has been provided for three well known disulphide-containing structures: *elastase*, *lysozyme*, and *thaumatin*. In case of UV-induced structural changes, RIP can be regarded as a sort of isomorphous replacement where the dataset after exposure has changed a electron density between sulphide bridges in structure.

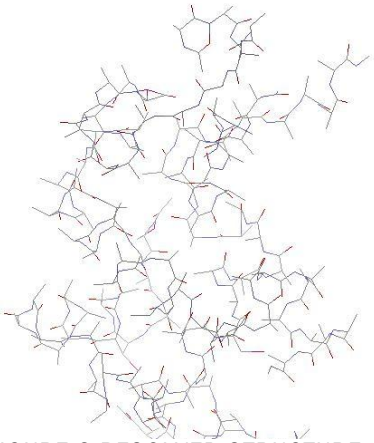


FIGURE 2 RESOLVED STRUCTURE OF LYSOZYME

Experiment

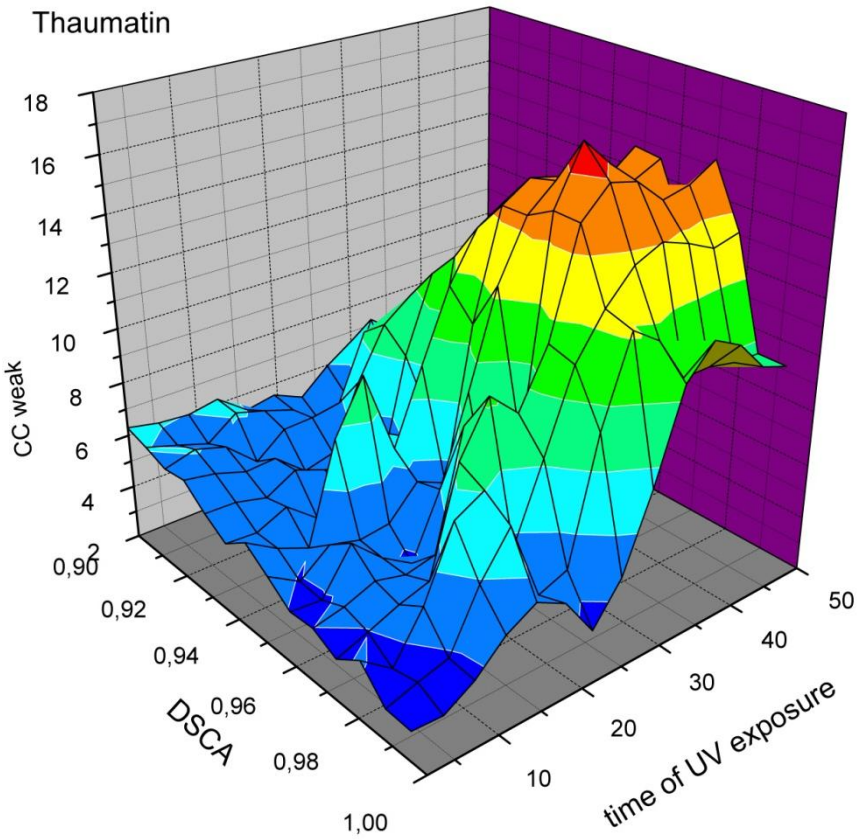
Data sets were collected by my supervisor on a third-generation synchrotron in Grenoble. Each protein was measured in three different modes of exposition to the UV light (2, 5, 10 minutes). In every mode 6 data were collected: one “before” exposition and 5 “after” with a different exposition time.

TABLE 1. DATA COLLECTION STATISTICS

	Elastase	Lysozyme	Thaumatine
Space group	$P2_12_12_1$	$P4_32_12$	$P4_12_12$
Unit cell parameters (Å) – before UV exposure	a=49.993 b=57.954 c=74.506	a=78.834 b=78.834 c=36.802	a=57.701 b=57.701 c=150.066
Resolution (Å)	45.750 - 1.450 A	45.750 - 1.099 A	45.750 - 0.722
Number of total reflections	39064	15887	46254

Calculations – CC weak

Heavy atom sites were determined by *SHELXD*. This calculations were made in order to find rules in correlation between optimal time of exposure and scale factor. Results of scaling can be found on fig. 3,4,5. The "after" data set was scaled by DSCA factor range from 0.900 to 1.000. Quality of data calculated can be easily estimated from correlation coefficient factor. It should be remembered that simple rule says that for CC weak factor grater then 8, structure can be solved with the Patterson method.



Results – CC weak

From graphs CC weak vs DSCA factor in time domain ones can find that there is correlation between time of UV light exposure and CC weak factor. It strongly depends on used protein. In two cases – thaumatin, lysosyme - when time of exposure grows also CC weak factor, for trials, grows which can be observed from 3D graphs.

FIGURE 3 CC WEAK FACTOR AS A FUNCTION OF DSCA FACTOR IN TIME DOMAIN OF UV EXPOSURE FOR THAUMATINE

For elastase it looks the same but after 20 minutes of UV exposure, protein probably is being damaged and because of that CC weak factor drops down.

Calculations – Standard deviation

Phasing and density modification was made after scaling. With the help of f2mtz, mapman standard deviation of density map was calculated. The standard deviation factor is better to evaluate quality of a density map.

Results – Standard deviation

Three graph from figure 6 shows standard deviation as a function of DSCA factor. Similarity to CC weak results can be observed on each graphs. In case of thaumatin correspondence is quite large and shows that even quality of the maps with lower CC weak value are satisfactory. For elastase similarity of graphs is also huge but position of peak indicates that this proteins should not be left for longer exposure of UV light because of its damages. Correlation in lysozyme and thaumatine case indicates that longer, about 20-50 minutes, UV light exposure might be optimal in RIP method.

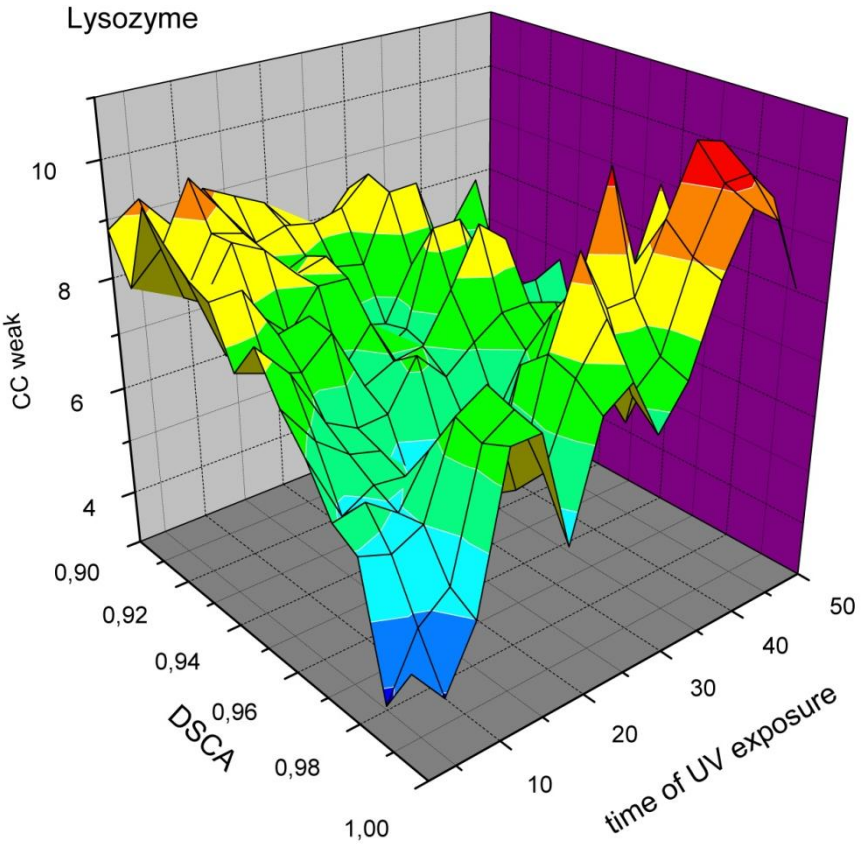


FIGURE 4 CC WEAK FACTOR AS A FUNCTION OF DSCA FACTOR IN TIME DOMAIN OF UV EXPOSURE FOR LYSOZYME

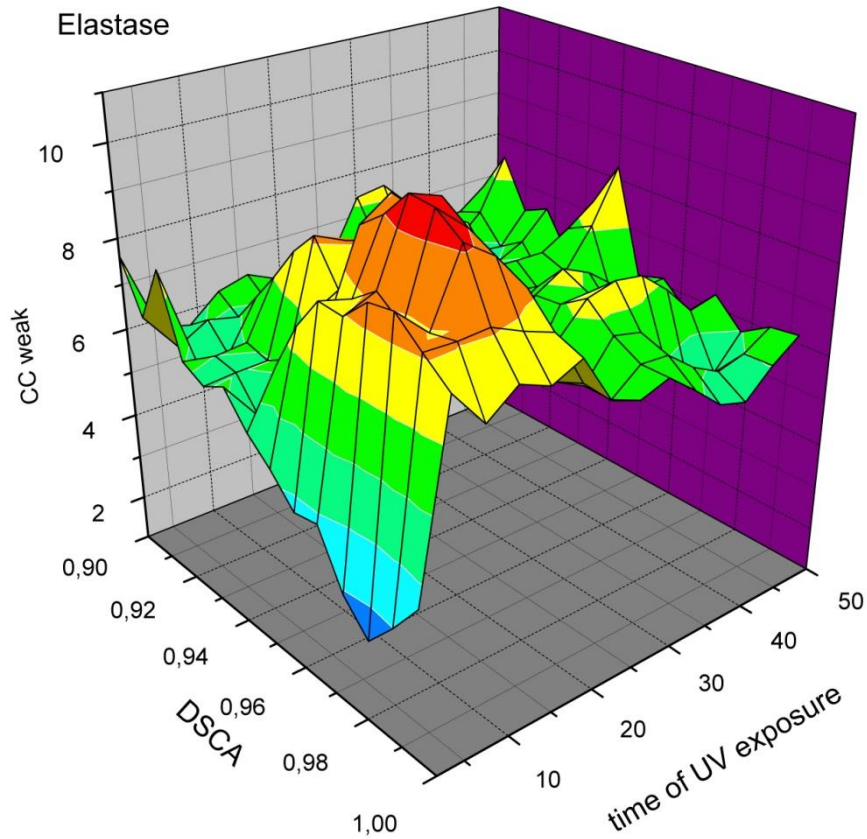


FIGURE 5 CC WEAK FACTOR AS A FUNCTION OF DSCA FACTOR IN TIME DOMAIN OF UV EXPOSURE FOR ELASTASE

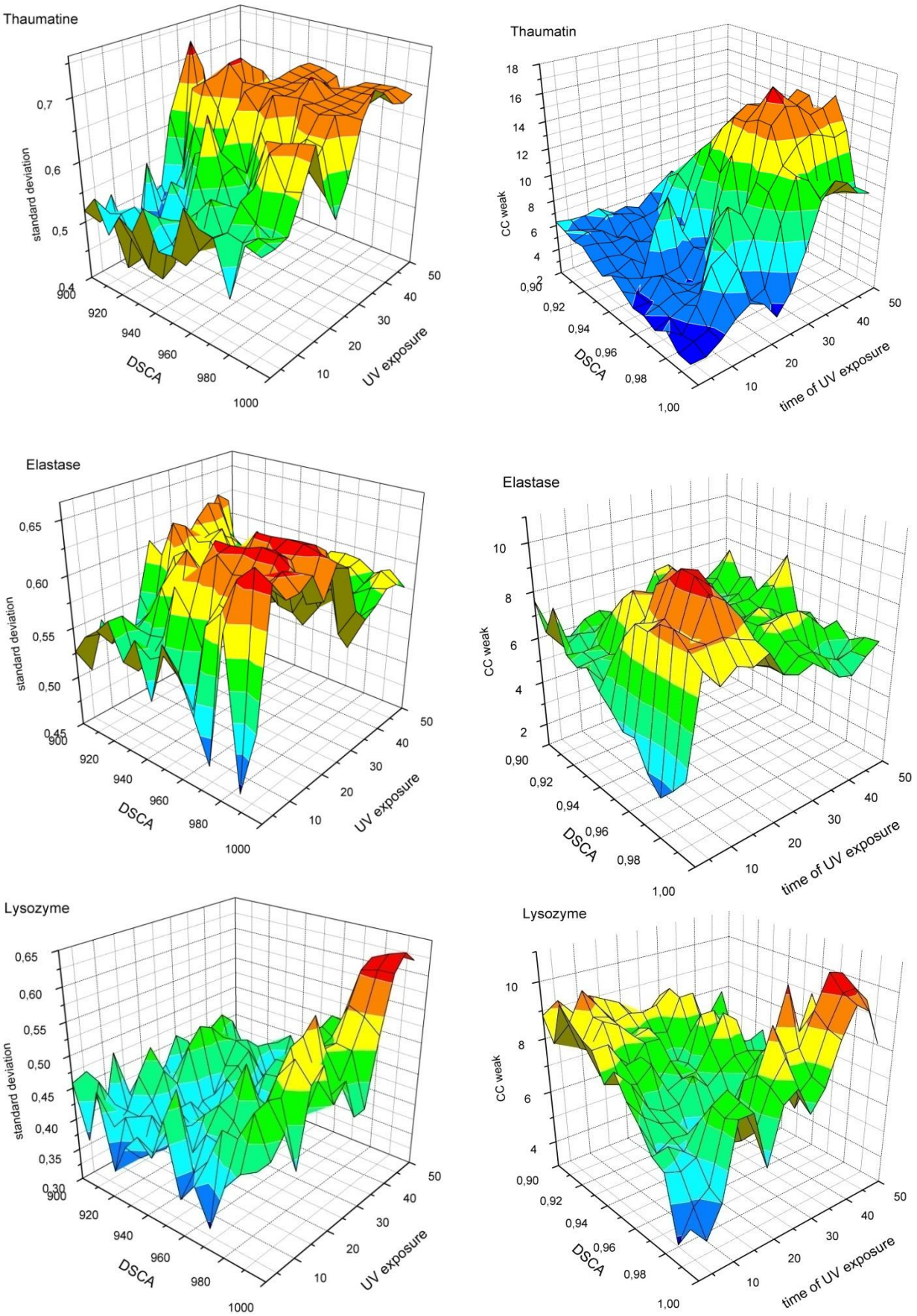


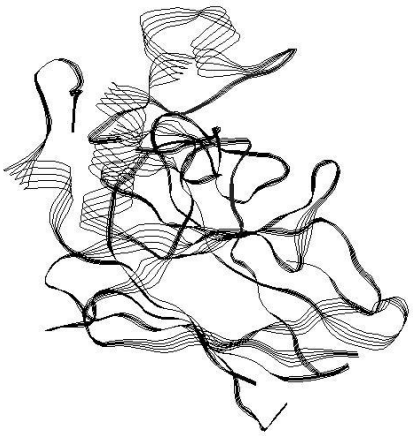
FIGURE 6 EVALUATION QUALITY OF DENSITY MAPS WITH CC WEAK FACTOR AND STANDARD DEVIATION

TABLE 2. DATA MAXIMUMS AND OCCURE VALUES OF DSCA AND TIME

	Standard deviation	DSCA	Time of exposure
Lysozyme	0,6148 – 0,6134	0,986 - 0,974	20 - 50
Thaumatine	0,697 – 0,696	0,934 - 0,953	30 - 50
Elastase	0,653 – 0,651	0,906 - 0,947	15 - 25

Structure of each protein has been calculated with best parameters (fig. 1,2,7).

References



Max Nanao, Raimond B.G. Ravelli Phasing Macromolecular Structures with UV-Induced Structural Changes. Structure 14, 791–800, April 2006

G.M. Sheldrick (2002), "Macromolecular phasing with SHELXE", Z. Kristallogr. 217, 644-650

T.A. Jones & S. Thirup (1986). Using known substructures in protein model building and crystallography. EMBO J 5, 819-822.

T.A. Jones (1992). A, A set of averaging programs. In "Molecular Replacement", edited by E.J. Dodson,

G.J. Kleywegt & T.A. Jones (1994). Halloween ... Masks and Bones. In "From First Map to Final Model", edited by S. Bailey, R. Hubbard and D. Waller. SERC Daresbury Laboratory, Warrington, pp. 59-66.

R.J. Read & G.J. Kleywegt (2001). Density modification: theory and practice. In: "Methods in Macromolecular Crystallography" (D Turk & L Johnson, Eds.), IOS Press, Amsterdam, pp. 123-135.

G.J. Kleywegt, M.R. Harris, J.Y. Zou, T.C. Taylor, A. Wahlby & T.A. Jones (2004). The Uppsala Electron Density Server. Submitted.

Kleywegt, G.J., Zou, J.Y., Kjeldgaard, M. & Jones, T.A. (2001). Around O. In: "International Tables for Crystallography, Vol. F. Crystallography of Biological Macromolecules" (Rossmann, M.G. & Arnold, E., Editors). Chapter 17.1, pp. 353-356, 366-367. Dordrecht: Kluwer Academic Publishers, The Netherlands.

Collaborative Computational Project, Number 4. 1994. "The CCP4 Suite: Programs for Protein Crystallography". Acta Cryst. D50, 760-763.

Appendix – plots

Figure 8. CC weak factor as a function of DSCA factor in time of uv exposure for thaumatine.

Figure 9. CC weak factor as a function of DSCA factor in time of uv exposure for lysozyme.

Figure 10. CC weak factor as a function of DSCA factor in time of uv exposure for elastase.

Figure 11. CC weak factor as a function of st.dev. factor in time domain of uv exposure for elastase.

Figure 12. CC weak factor as a function of st.dev.factor in time domain of uv exposure for lysozyme.

Figure 13. CC weak factor as a function of st.dev.factor in time domain of uv exposure for thaumatine.

FIGURE 8 CC WEAK FACTOR AS A FUNCTION OF DSCA FACTOR FOR THAUMATINE

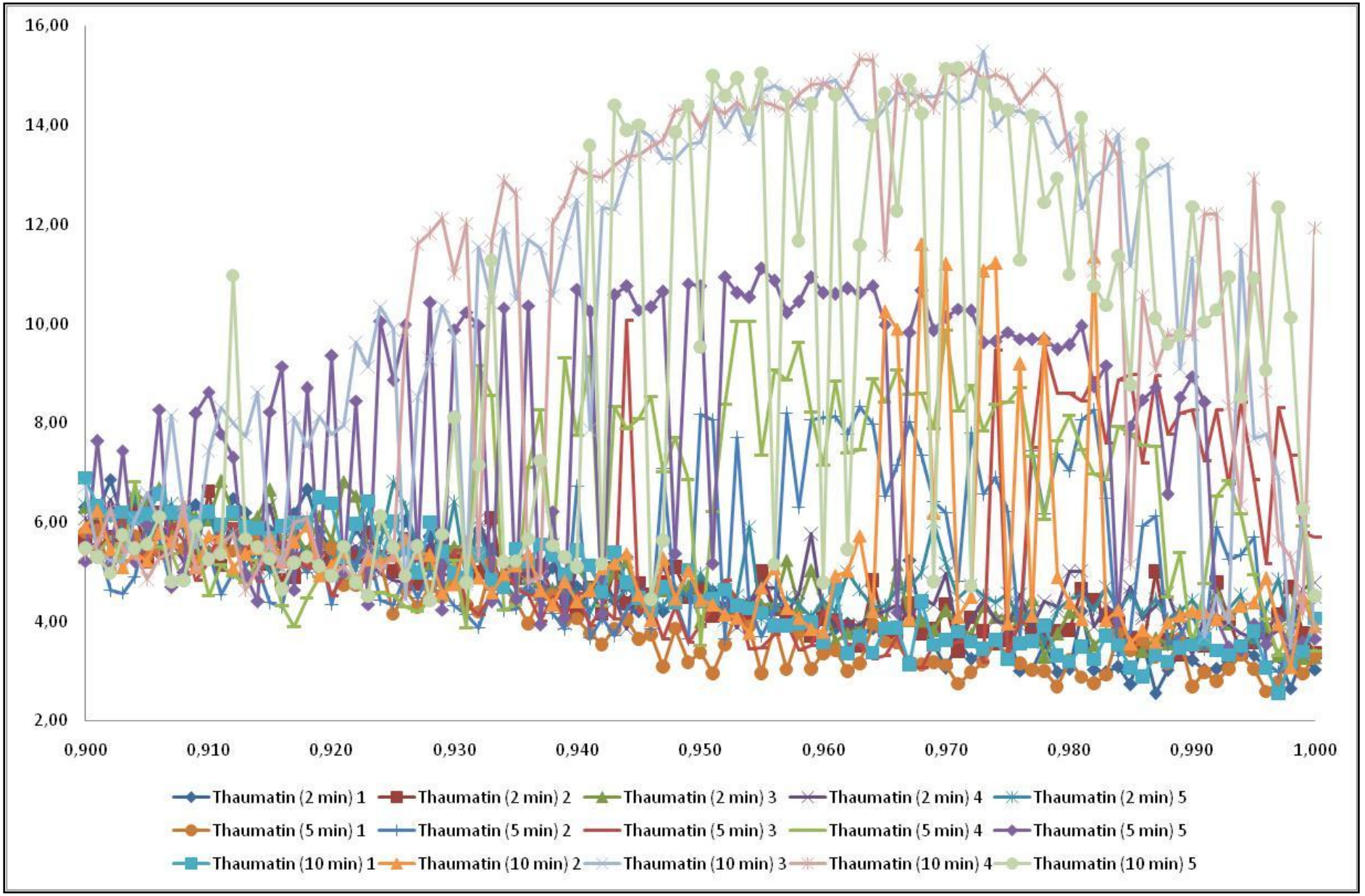


FIGURE 9 CC WEAK FACTOR AS A FUNCTION OF DSCA FACTOR FOR LYSOZYME

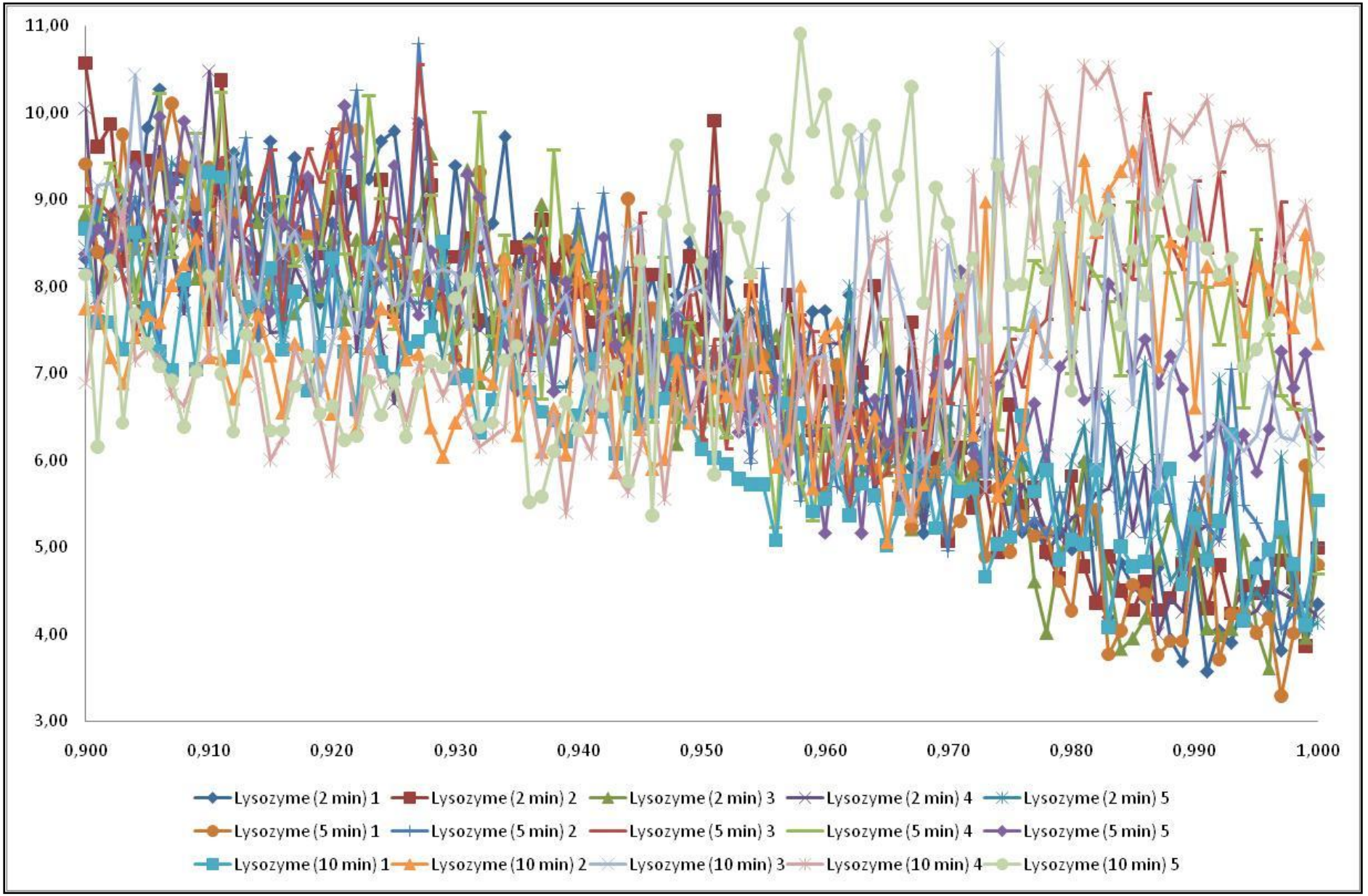


FIGURE 10 CC WEAK FACTOR AS A FUNCTION OF DSCA FACTOR FOR ELASTASE

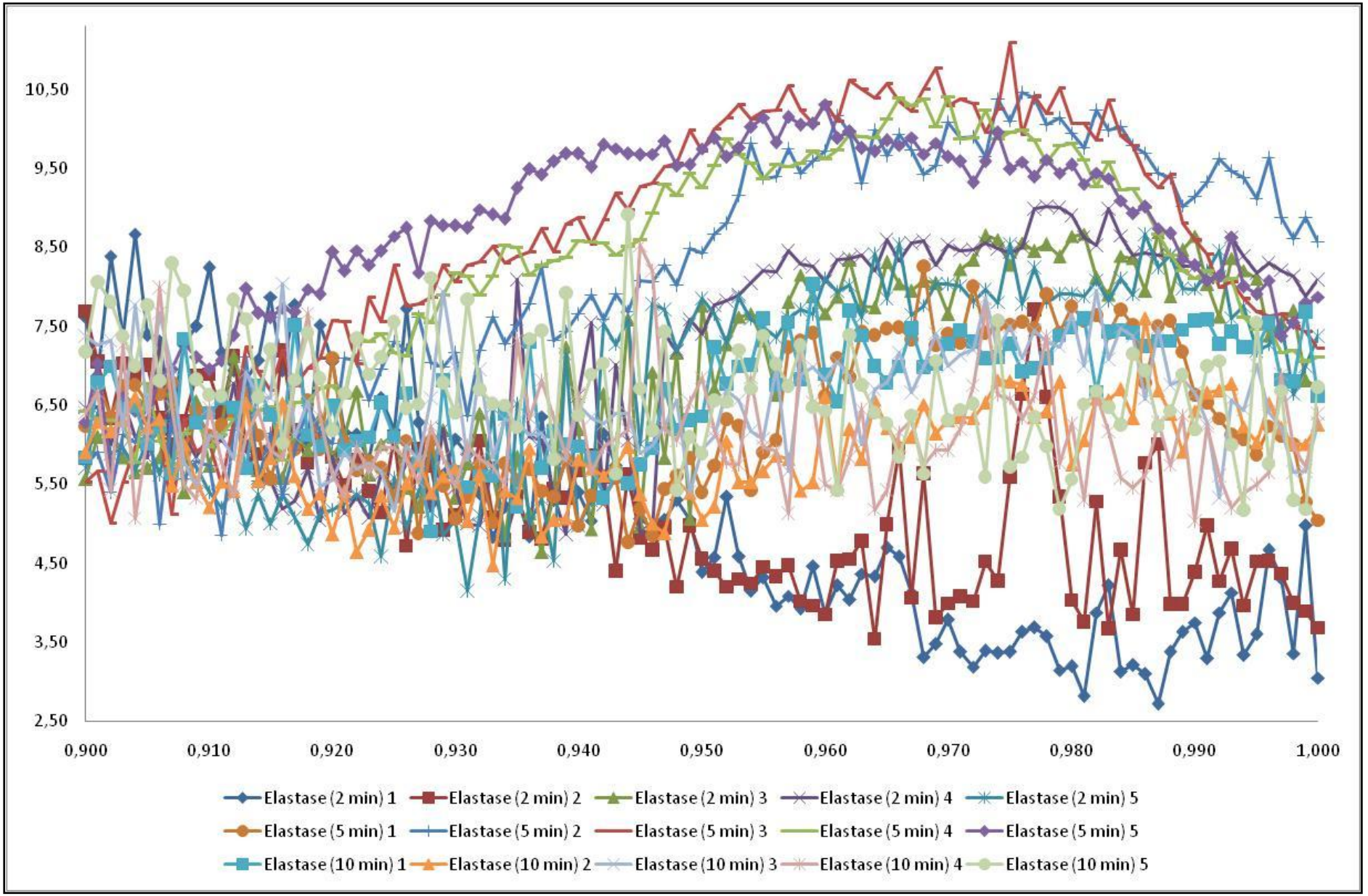


FIGURE 11 CC WEAK FACTOR AS A FUNCTION OF ST.DEV. FACTOR IN TIME DOMAIN OF UV EXPOSURE FOR ELASTASE

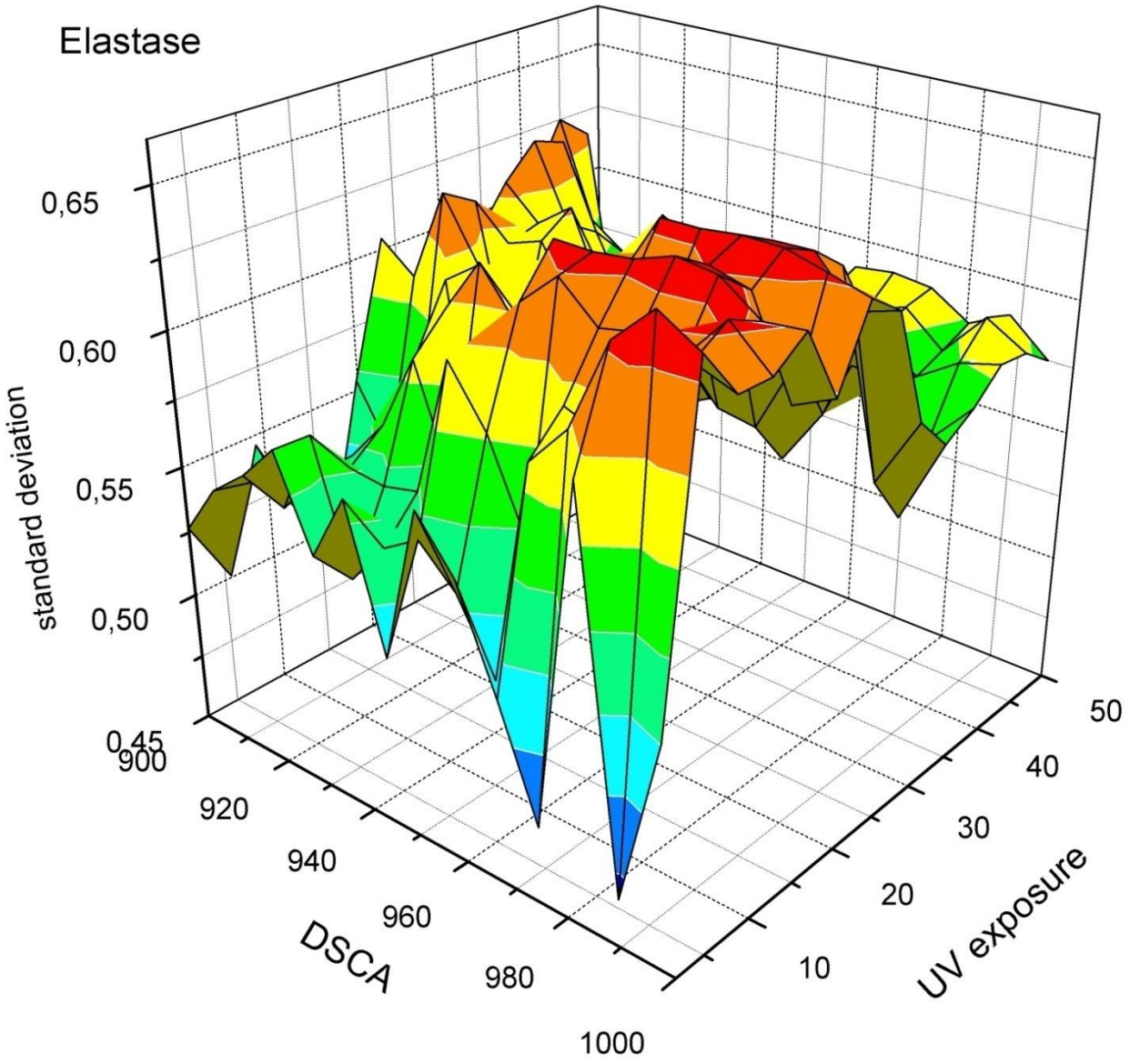


FIGURE 12 CC WEAK FACTOR AS A FUNCTION OF ST.DEV. FACTOR IN TIME DOMAIN OF UV EXPOSURE FOR LYSOZYME

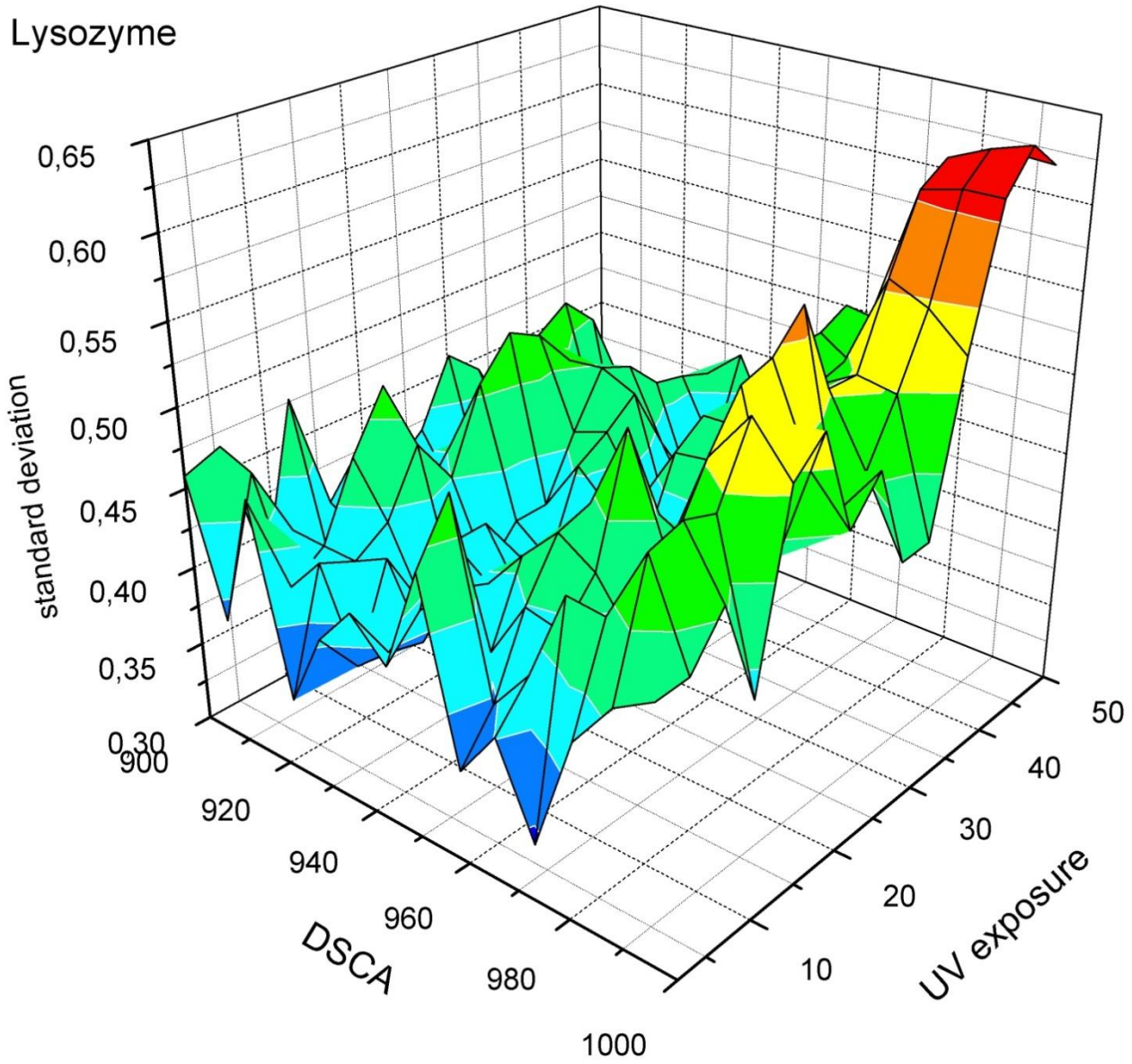


FIGURE 13 CC WEAK FACTOR AS A FUNCTION OF ST.DEV. FACTOR IN TIME DOMAIN OF UV EXPOSURE FOR THAUMATINE