



## Biometric Estimation for Understanding the Nature of *Vorticella* Stalk Contraction-Extension Repeated Consequential Cyclic Processes

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**Abstract:** *Vorticella* stalk is the storehouse of two types of novel proteins, known with the names of *spasmins* and *batonnets*. On the basis of nature of proteins and the arrangements of amino acid residues of these proteins, the repeated consequential cyclic processes of contraction dynamics worked by neutralizing the negatively charged amino acid residues as per the laws obeyed by Heber-Weiss, Nernst and Fenton reactions.  $H^+$ -integrated physiological performances in combination with pCa (partial pressure of calcium ion concentrations) and DNFB (2,4 – dinitrofluorobenzene/Sanger's reagent) concentration gradients at the range of 1mM to 5mM represented velocity inclination in acidic medium which were more actively pronounced if it was compared with alkaline medium where permeabilized stalk exaggerated potential biochemical-shift-perturbation if it was in respect of non-permeabilized live specimens in both artificial as well as in natural medium in different experimental trials under controlled electro-physiological instrumental setup conditions. On the basis of these experimental designs it was confirmed that *spasmins* and *batonnets* are two different types of novel proteins with multitudes of potential applications in the favour of biomedical engineering devices formulation, then their construction at the nano-scale where  $H^+$ -integrated pCa dependent electrophysiological nature of recommended proteins were found more ROS (reactive oxygen species) resistant if there was the introduction of DNFB in fixed concentrations than in the acto-myosin as well as tubulin-dynein systems being exclusively controlled under post-translational biochemical reactions catalysed in the light of software based modern bioinformatics' tools and techniques. In live as well as permeabilized specimens, different types of biochemical reaction kinetics of amino acid residues were performed at different rates among the sequentially determined *spasmins* and *batonnets* like novel proteins where molecular orientations and motive-force generation in measurable parameters per millisecond confirmed the electrophysiological significance of *Vorticella* stalks' on the basis of colligative nature of novel proteins of saccular compartments of spasmoneme, the well explained active contractile organelle of the stalk in relation with other resembling proteins found in Protein Data Bank (PDB) as *centrins*, *calmodulins* and others significantly pronounce their life saving and medicinal properties. This present statement/study was aimed to know the biochemical behaviour of *spasmins* and *batonnets* like novel proteins in the light of electrochemical behavior of the *Vorticella* stalk under some selective chemical stress conditions, that's why this research helped us to know the ROS resistance properties of novel protein polymers found in stalk. On the basis of which reference proteins as described in this paper can be used as a diagnostic tools in pharmaceutical industries in the favour of molecular medicines and drugs' designing.

**Keywords:** *Vorticella*, Stalk, *Spasmins*, *Batonnets*, DNFB, pCa.

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## I. INTRODUCTION

Among all known contractile devices, the *Vorticella* stalk is fastest known which works on the basis of negatively charged amino acids neutralizations, just like the behavior of gelatinous proteins as in the electrophoretic medium of experimental induction. *Spasmins* and *batonnets* are novel known chief contractile proteins in *Vorticella* lobular *sacculles* inside the spasmoneme oriented in their opposite facial directions of one another where *spasmins* dispersed irregularly and *batonnets* in peripheral confinements that of spasmoneme in crescent forms viewed in cross-sections of stalks at the level of microscopic morphological and anatomical descriptions of *light/optical and electron-micrographs (L/O&EMs)* under semitransparent gelatinous cytoplasmic as well as *fibrillar-matrices* enclosed in two distinct compartments in the form of ever active organelle designed to accomplish contraction dynamics of the stalk in association with structural associates as other sub-cellular organelles out-pouched during stalk formation. The spasmoneme is contractile in nature due to the presence of *spasmins* (20 kDa fibrous protein with irregular orientations) and *batonnets* (globular proteins with regular helical symmetric orientations).<sup>1</sup> Bird's foot like structural organizations of associated structures of micro- and macro- molecules as very tough and complexes structural organizations in symmetric forms reveals more difficult routes of energy signal transduction propagating along the lengths of fragile and multidirectional globular as well as filamentous proteins as F/G-actins which are in actions in repeated cyclic consequences connecting ER, *myonemes* and surface membranes for electromotive force and power generation through systematic unknown defined paths for ionic travels/jumps reflecting electromagnetic induction to facilitate the continuation of contraction dynamics in a repeated consequential unbreakable manner where T – tubules distribution was same in *Vorticella* stalks as well as in frogs' and humans' skeletal muscles endoplasmic reticular resemblances including smooth and cardiac muscles too.<sup>2</sup> Torsion-detorsion rotational dynamics of *Vorticella* stalk along the length from the base to *scopula* (the point where stalk gets attached with zooid) in a bending pattern due to the presence of specialized molecular orientations, characterizations by *spasmins*, *batonnets* and most of the novel known and unknown proteins dispersed in gelatinous matrices of different compartments playing their life supporting biochemical roles for specimens survival<sup>3</sup> revealed by western blotting including *anti-spasmins* as anti-N-terminal molecular marker for *spasmins* as well as *VcCenPB* anti-N-terminal in the same way for *centrins* as molecular markers for molecular diagnosis of *spasmins* by leaving the focus on *batonnets* as per most of the recent scientists of this time. DNFB well-known metabolic uncoupled produces ROS (reactive oxygen species) in the reactivation medium in the form of free radicals of fluoride to reflect diagnostic properties of proteins against ROS generation per mole for reflecting antagonistic properties against bio-molecular-chemical-kinetics in the form of chemical-shift perturbation, thus exerted poisonous effects resistances against ROS generation for *spasmins* and *batonnets* in respect of molecular folding dynamics under the hindrances of three-dimensional molecular orientation model revelation at the level of *nuclear magnetic resonance (NMR)*, *X-ray crystallographic data interpretations and the molecular docking* like beneficial experiments on one hand

whereas on associative cooperative hands DNP (dinitrophenyl) derivative production revealing sequencing providing primary levels of information at the level of amino acid functional properties continues contraction-extension cyclic repeated processing by opposing metabolic paths and processes in the form of second-order of reaction-kinetics as a therapeutic reagent in reactivation media/mM of molecular gradients in *Nernst's*, *Fenton* and *Heber-weiss* reaction kinetics. Here in these cases, biochemical reaction kinetics is out of hindrances due to opposing chemical equilibrium shifts conditions in a regular mode, quantified as chemical-shift perturbation.<sup>4</sup> This present study/statements as described in this paper helped to understand the biochemical nature of *spasmins* and *batonnets* like novel proteins, and thus on the basis of information available in this paper further research proposal can be designed for higher level of information on the basis of proteomics and metabolomics which will definitely revolutionize the products of pharmaceutical industries in the same/different way as we see findings related with *CRISPER-Cas9*/other related/non-related proteins available at the sites of *Protein Data Bank (PDB)*.

## 2. MATERIAL AND METHODS

### 2.1 Cell culture

The live specimens of *Vorticella* were collected from seasonal shallow water pond bodies from Chapra city Bihar in India. These ponds were good places for specimen survival and the sample collections for the purpose of studies and research due to intoxication of pond water. Reason behind it was that the specimens were good ecological indicators. Their presence in optimum amount, found fixed with twigs and branches of *Chara*, *Nitella* and *Myriophyllum* indicated good environmental conditions with clean and clear vision for sample identification and their collections during winter season (December to January, 2010 - 2014) upto 3 to 4 kms of range. Specimens were collected throughout the years from that places when needed.<sup>5</sup>

### 2.2 Essential equipments used in experiments

Glassware used in the experiments were Petri dish, watch glasses, beakers, pipettes, bottles and jars of different sizes (big, small and medium according to need) of Borosilicate company available in laboratory.<sup>6</sup> Single electrode pH meter of Systronic (MK-IV, Naroda Sl. Number 6936), Wensar chemical weighing machine model number ECB300, Microscopes – Magnus MS 24/13, Olympus India Pvt. Ltd. and Olympus ch2ibimf, Olympus India Pvt. Ltd. New Delhi, India. Photo-video-graphic camera – Nikon 12.1 150 3200 P/S/A/M (coolpix) along with its attachment device was used for photography and video recording. Computers were used for *Vorticella* stalk contraction data analysis and their presentation.<sup>7,8,9</sup>

### 2.3 Clinical procedures in experiments

The specimens collected from nearby seasonal ponds were cultured in *natural pond water (NPW)* of the same pond from where specimens were collected and *artificial pond water (APW)* prepared in laboratory by mixing 0.1 mM of NaCl, KCl and CaCl<sub>2</sub> for each of the chemicals of equal strengths in equal proportions. After every two days of incubation periods newly prepared culture media with food supplements (paste of egg yolk in 1/4 proportions for each newly prepared medium were used) were used for specimen survival. For

pray-predation interaction prevention in NPW, first of all NPW was filtered with filter paper to avoid biotic interaction for cultural existence of the isolated *Vorticellids*. For APW preparation, double distilled water was prepared in the laboratory by using a distillation machine on one hand. On another hand for experiments to know effect of  $[H^+]$ , pCa and DNFB from 1 mM to 5 mM, distil water were purchased from local scientific chemical supplier of the city (Chapra, Bihar) then APW was prepared to prevent other biochemical interaction with existing chemicals of NPW and of tap water. All the experiments were done in good laboratory conditions at 25 to 30°C of temperature. Throughout the years, specimens were maintained in laboratory in big glass-jars where there was optimum sunlight present throughout the years (2010 – 2014) from March to February of academic calendars.<sup>10</sup> For experimentation,  $[H^+]$  from  $14.81 \times 10^{-10}$  to  $7.12 \times 10^{-10}$  moles were used by using buffer tablets of fixed pHs of Merck Company purchased from biochemical laboratory suppliers for acidic and alkaline balance rectification and calibration for pH meter membranes permeability accuracy reading testing of pH meter of Systronic company (Model number - MK 4) from Ahmedabad (Sl. No. 6936). DNFB (2,4-dinitro-fluorobenzene) available in laboratory was used in the strengths of 1 mM to 5 mM after its solution preparations in different strengths in consequences of 1, 2, 3, 4, and 5 to know their effects on velocity profiles in reference with  $[H^+]$  in comparative account of live and Tx-100 (Tx) treated (less than 1% v/v) and untreated bio membrane permeabilization conditions for *Vorticella* stalk contraction bio chemical shift perturbation reflected in the format of shaded areas of the figs. 1 & 3 in respect of smooth diagrammatic representation of time-intervals by insignificant bumpy omissions in both conditions (alkaline as well as in acidic conditions per trial) rather than bumpy illustration for conclusive perfection rather in the forms of figs 2 & 4 (with insignificant bumpy illustration omissions per trial) respectively. The reactivation media as a support system included 1% Tx-100 at pH 7.0 in combination with 20 mmol/l KCl, 10 mmol/l EDTA and 10 mmol/l of Tris-maleate were used as a wash solution. The extracted specimens then were washed for three times in a washing medium containing 50 mmol/l KCl, 2 mmol/l EDTA and the 10 mmol/l of Tris-maleate at neutral pH (7.0) and then the specimens were kept in 1% Tx-100 again, later on DNFB and pCa were used in the concentration of media containing 50 mmol/l KCl and 10 mmol/l of Tris-maleate buffer at pH 7.0 at neutral solution at room temperatures from 25 to 30°C.<sup>11,12</sup>

## 2.4 Clinical considerations

To know the effects of acidic and alkaline solutions from pHs 5.5 to 7 and 7 to 9 respectively, HCl and KOH were used after finding accuracy measurement quality testing and its conformity of pH meter reading rectification rechecking for obtained data significance in respective ways. For testing accuracy of pH meter, different strengths of buffer tablets from pH 3 to 14 were used, where

reversibility of contraction-extension biochemical cyclic kinetics of *Vorticella* stalks contraction dynamics were confirmed from pHs 5.5 to 7 more pronouncedly than in alkaline solutions from pHs 7 to 9 referred proto osmotic support evidences in *protonation-deprotonation* (favoured by  $H^+$  and  $Cl^-$  ionic concentrations of reactivation media supporting/agonizing pCa gurgitation/regurgitation reaction kinetics at the level of ryr of linkage-complexes on one hand whereas *spasmins* and *batonnets* folding kinetics at the level of higher order molecular organizations on another way still waiting bioinformatics illustration) *Henderson-Hasselbalch* second-order of reaction-kinetics supported by thermodynamic wave signaling as frequency and duration profiles in terms of  $\Delta x/\Delta t$ , where  $\Delta x$  indicated distance travelled by the cell body/zoid in frequencies per minute in respect of time intervals  $\Delta t$  in per second in respect of Hill's parameter 0 to 1.<sup>13</sup>

## 3. STATISTICAL ANALYSIS

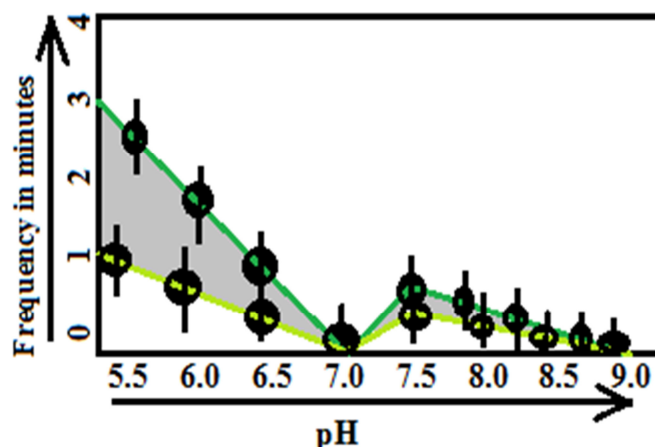
### 3.1 Bio-statistical Analysis and Software utilizations

The biometric calculations were performed after data transfer from camera to computer by using biometric formulae of mean and standard deviation in terms of Stock's charts of low, medium and high range of data arrangement and precipitation in terms of tables then graphs/figures as in this manuscript fig. 1 to 4 in a comparative account in Tx-100 treated (upper case in figs. 1 & 3) and untreated (lower case in figs. 1 & 3) conditions in respect of time intervals  $\Delta t$  (figs. 2 & 4) (in minutes for frequency and in seconds for duration) by using Windows 7 basic, Microsoft Excel and paint software.<sup>14</sup> These steps and software used during research helped in bio-statistical data analysis, graphics presentation and the model development (figures – 5 & 6).

## 4. RESULTS AND DISCUSSIONS

### 4.1 $H^+$ - integrated pCa experimental outcomes on Tx-treated versus untreated live specimens

$[H^+]$  gradients generated positive charges into the surrounding medium when introduced. The protic potentials generated by protonation of  $[H^+]$  around the cationic R-grouped amino acid residues of *spasmins* and *batonnets* of spasmoneme of the stalk brought modifications in molecular orientations along the axis of x, y and z in terms of birefringence as on Snell's law in respect of protic affinities of the surrounding medium.<sup>15</sup> Electrostatic forces of attraction generated in respect of concentration gradients of negative logarithms of protons of the cytoplasmic matrix of spasmonemal *sacculs* inside the *Vorticella* stalk around proteins confinements. Charge neutralization of cationic functional grouped amino acid residues undergone sifts perturbation as in molecular orientations through displacements in electronic shifts of power potentials in omega ( $\omega$ ) of *protonation-deprotonation potentials displacements* and it was 30 to 50% more pronounced in the presence of Tx-100 by means of frequency and duration profiles (figures 1, 2 and table 1).<sup>16</sup>



**Fig – 1: Effects of Tx-100 (upper case) treated and untreated (lower case) on velocity profiles in minutes of *Vorticella* stalk contractility from  $14.81 \times 10^{-10}$  to  $7.12 \times 10^{-10}$  moles of  $[H^+]$  (Table I).**

**Table – I: Effect of pCa concentrations from 1mM to 5 mM on Triton x-100 treated *Vorticella* stalk for contraction measurement in milliseconds (ms) for molecular binding affinities establishments in spasmonemal cytoplasmic solutions when Fick's law of diffusion was in active state in permeabilized specimens than the live specimens**

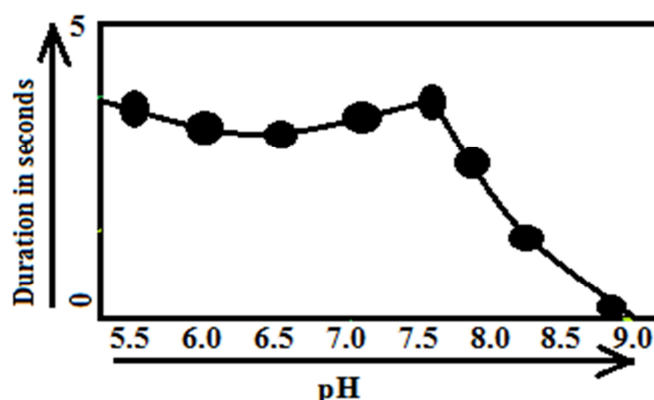
pH	pCa 1mM	pCa 2mM	pCa 3mM	pCa 4mM	pCa 5mM
5.5	9.14	8.57	7.55	6.53	5.86
6.0	8.43	8.23	6.58	6.26	4.59
6.5	7.25	7.53	7.42	5.51	5.45
7.0	6.57	6.59	6.73	6.37	4.81
7.5	5.52	5.41	5.35	5.42	5.78
8.0	5.66	4.82	4.24	4.48	4.24
8.5	4.38	3.54	7.67	3.93	3.67
9.0	3.71	2.61	2.16	2.50	2.13

(S.D.= 0.01, N = 5)

#### 4.2 Biophysical-experimental integration on proteins' performances of the given specimens

Torsion-detorsion rotational dynamics of amino acid residues of the stalk for known amino acid residues sequences of *spasmins* & unknown amino acid residues sequences of *batonnets* worked on the basis of their radius of gyration as per the lengths of bending and stretching bond angles in respect of *Ramachandran plots*<sup>17</sup> more strictly along with *Levinthal paradox*<sup>18</sup> which is for bioenergetics estimation of catalytic reactions as receptor-ligand associative performances of protein folding dynamics for their functional sustainability<sup>19</sup> in non-equilibrium reaction kinetics for thorough repeat of contraction dynamics in respect of centripetal and centrifugal forces which generates tensions around microfilaments of F – and/or G – actins ( *spasmins* and *batonnets* as in stereo-ciliary beats at the apex of hair cells of internal ears) in respect of thermodynamics.<sup>20</sup> The amino acid sequencing and Raman's spectroscopic investigations<sup>21,22</sup> in *Vorticella* stalks' contraction dynamics on the basis of frequency profiles of protein-protein interactions at the levels of surface bio-membranous attachments of spasmoneme in saccular compartments can be exclusively

used for the study to know the importance of *spasmin* proteins in the process of bio-implants formulations, bio-immunoassay related diagnostic-therapeutics as well as biosensors' qualitative advancements<sup>23</sup> for health related instrumentation up-gradations on the basis of species specific photon related hydration/dehydration confinements for stereoscopic/optical-densitometry irrational collapse hindrances managements and their competitive adsorption-desorption behavioral performances of the related molecules under the medium of existence in the same way as in proto-osmosis/electro-osmotic phenomena occurs in electromotive induction and thus we can understand spectral features of *spasmins* and *batonnets* on the basis of frequencies profiles of amino acid residues in the fields of biomedical utility of novel proteins in the light of triton x-100 treated conditions which is more accurately than the live specimens/samples case studies either in vivo/in vitro conditions of the given samples' electrophysiological characterizations in different media conditions of versatile colligative nature in different experimental trials possibilities as the case occurs in this graphic-tabular defined-cum-analysed presentations (figures 1, 2 and table 2).



**Fig – 2: Duration of Tx-100 treated *Vorticella* stalk in seconds from  $14.81 \times 10^{-10}$  to  $7.12 \times 10^{-10}$  moles  $[H^+]$  gradients, both independent and in association of  $Ca^{++}$  concentration gradients from 1 mM to 5 mM (Table - 2).**

**Table – 2: Effect of pCa concentrations 1 mM to 5 mM on Tx-untreated *Vorticella* stalk contraction in milliseconds (ms) in terms of binding kinetics (millimole/ms) and degree of rotation of stalk,  $6+1/2$  in numbers with 0.5 level of significance (including fixed notation of phi ( $\phi$ ), psi ( $\psi$ ), theta ( $\theta$ ), omega ( $\omega$ ) and tau ( $\tau$ ) for *spasmins* and *batonnets* in primary stretched states of polymeric proteins) in live specimens**

pH	pCa 1mM	pCa 2mM	pCa 3mM	pCa 4mM	pCa 5mM
5.5	5.01	4.81	4.08	3.50	2.22
6.0	4.80	4.64	3.54	3.00	1.50
6.5	4.63	4.22	3.07	2.50	2.30
7.0	4.40	3.53	2.63	2.10	1.50
7.5	4.21	3.14	2.36	1.60	1.20
8.0	3.80	2.82	2.02	1.30	0.50
8.5	3.55	2.43	1.55	1.20	0.25
9.0	3.13	2.11	1.01	0.50	0.00

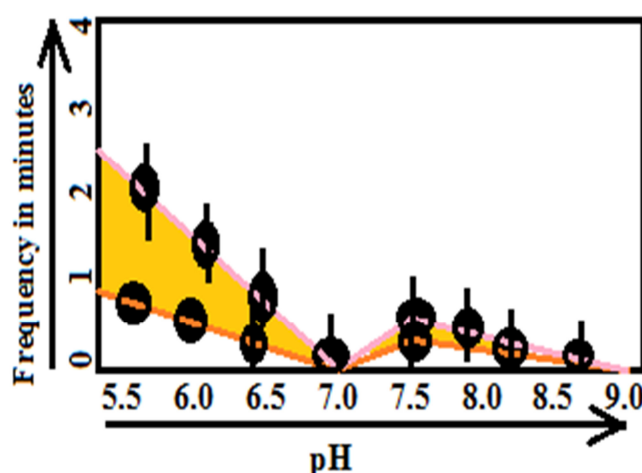
(S.D. = 0.01, N = 5)

#### 4.3 $H^+$ - dependent pCa and DNFB effectiveness on electrochemical behavior of protein folding kinetics

$Ca^{++}$  concentrations in the presence of  $[H^+]$  in both along with Tx-100 treated and untreated conditions in the *Vorticella* stalks generates depolarized protic potentials (amplitude 74 to 3.8 mV) and thus generated significant velocity profiles in respect of molecular infusions and diffusions by Fick's law.<sup>24</sup> DNFB in media infusion conditions in respect of  $Ca^{++}$  effectiveness molded bioenergetics protein binding and folding kinetics from 2 to 5% positive effectiveness on the biochemical nature modifications through the rate of amino acids residues orientation sifting per ms. These investigation shows antagonistic nature of *Vorticella* stalk if it was compared with *actomyosins* of bell and the frogs and human skeletal muscles biodynamics including ciliary and flagellar<sup>25</sup> and the axonemal performances due to dynein arms exhibition rather than Sfilp and Bcl2<sup>26, 27</sup> like protein polymers and thus we can say that *spasmin* is specialized F – and G – actins worked on the principles of *centrins* and *calmodulins*, but not *batonnets* another analogue and supportive system of contraction dynamics in the stalk, which is a globular protein worked in the same way as *actomyosin* in *myonemes*. *Spasmin* is an electrogenic putative polymer represents proto-osmotically induced performances<sup>28</sup> with highest bending abilities due to the presence of tyrosine residues<sup>29</sup> which is still not confirmed in *batonnets* but cooperative nature says analogy rather than homology of *spasmins* and *batonnets* rather than any in-depth correlative account with *actomyosin* reaction kinetics on the basis of

distinct line of genomic evolution of macro and micro molecules.<sup>30</sup> *Spasmin* has more similarities with actions, *centrins* and *calmodulins* on the basis of folding and binding kinetics of negatively charged groups of amino acid residues in second order of reactions as occurs in different types of *immunoglobulins* (heterodimeric classes in origin, evolution and functions as *IgG* - monomer, *IgA* - J-shaped monomer/dimer, *IgM* - J-shaped pentamer, *IgD* - monomer and *IgE* - monomer) and Bcl2 like protein polymers whereas *batonnets* follows perhaps sliding filament biomechanics (visual bio-dynamics in conjecture with *spasmonemal* association of *batonnets* and *spasmins* with *saccules* as *spectrins* of RBCs with variations in molecular orientations along with mode of biomechanical performances) as *actomyosin* rather than folding biomechanics<sup>31, 32, 33, 34</sup> under differential ROS conditions for DNP production and other metabolic inhibition of DNFB promoting antagonistic repressive stress conditions after prolonged treatments of 10 minutes in all or nothing electrophysiological fashions as in cardiomyocytes<sup>35</sup> (figures 3, 4 and table 3) as per hyper and hypo polarizations for differential vectorial conditions of bioenergetics structural modulations of *spasmins* and *batonnets* as per *calmodulins*, *spectrins*, *dynelectins*, *caltractines*, *actomyosins* and others (relative/non-relative groups of proteins) taking part in the biomolecular structural and functional modular operations (both qualitative as well as quantitative versions) for metabolomic based structural designing on the basis of receptor-ligand binding affinities of *spasmins* and *batonnets* as an alternate as per more ROS resistant drugs designing to prevent life threatening diseases

in the fields of biodynamics as per the alternative molecular medicines.<sup>36</sup>



**Fig – 3: Effects of DNFB after Tx-100 treated (upper case) and untreated (lower case) from 1 mM to 5 mM on Tx-100 treated *Vorticella* stalk contraction pattern in minutes (Table - 3).**

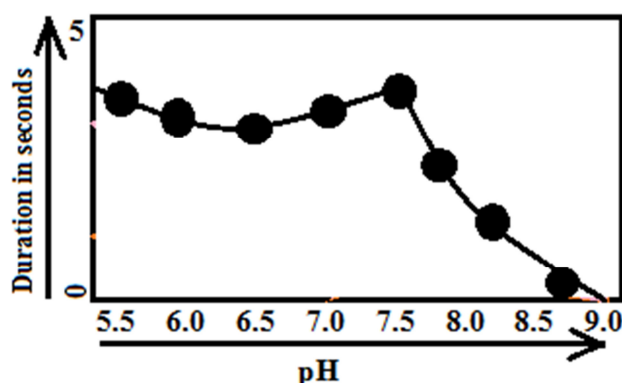
**Table – 3: Effect of DNFB concentrations from 1 mM to 5 mM in terms of ROS production on Triton x-100 treated *Vorticella* stalk contraction performance in association with percentage (%) of birefringence modifications in terms of molecular resistance (omega ( $\Omega$ )) in permeabilized specimens (S.D.= 0.01, N = 5).**

pH	DNFB 1mM	DNFB 2mM	DNFB 3mM	DNFB 4mM	DNFB 5mM
5.5	80.50	85.01	90.58	85.07	99.91
6.0	75.07	80.02	85.06	90.08	95.73
6.5	70.06	75.53	80.03	85.53	90.51
7.0	65.05	70.09	75.55	80.56	85.54
7.5	60.53	65.07	70.01	75.03	80.98
8.0	55.52	60.06	65.54	70.51	75.57
8.5	50.51	55.45	60.02	65.02	70.06
9.0	45.50	50.45	55.57	60.55	65.52

#### 4.4 Triton X-100 treated *Vorticella* stalks integral performances under the effectiveness of DNFB concentration gradients

Tx or Triton x-100 is for to enhance the molecular diffusivity by molding the molecular binding and interactive forces among the bio-membranous lipoproteins (with 1 to 5 % of carbohydrates) molecules in quasi-symmetrical alignments in hanged outs associative conformational native structural dynamics, hence, Raman spectroscopic analysis is a better platform for bio-electro-chemical analysis which is even better than in the fluorescent/radiological approach along with CCD (charged-coupled-device) detectors for reducing natural

aberrations for red/IR excitation at the range of 630 to 850 nm and thus, molecular polarization/birefringence of *spasmins* and *batonnets* can be better eluted in clinical conditions to establish correlations among the frequency aberrations among the Tx-100, *fluorochromes* and radioactivity based dynamic optimization in the light of *SQL*, *R* and *PYTHON* like programming languages fitting their data in terms of IR-FT feasibility to boost hypothesis, results and discussions at the ground level of amino acid residues as tyrosine, lysine, phenylalanine, tryptophan etc<sup>37</sup> in the light of optical densities and ROS and DNP productions by metabolic uncouplers as DNFB (figures 3, 4 and table 4).



**Fig – 4: Duration of Tx-100 treated *Vorticella* stalk under DNFB concentration gradients from 1 mM to 5 mM in seconds (Table - 4).**



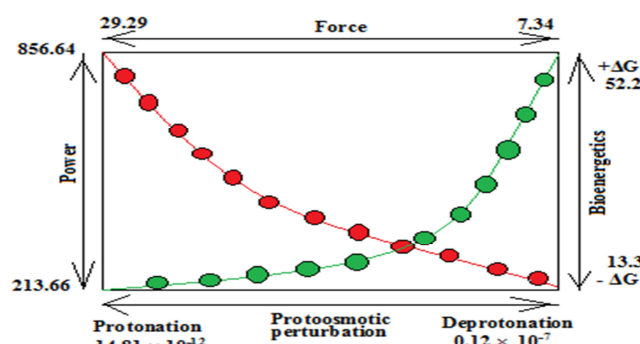
**Table – 4: Effect of DNP production in DNFB concentrations from 1 mM to 5 mM on Tx-100 untreated *Vorticella* stalk contraction in milliseconds (ms) estimation in Avogadro's number i.e. in multiplication of molar concentrations of  $6.023 \times 10^{23}$  for ryanodine receptors (ryrs) in terms of DNP (dinitrophenyl derivatives) amino acids production in live specimens (S.D.= 0.01, N = 5).**

pH	DNFB 1mM	DNFB 2mM	DNFB 3mM	DNFB 4mM	DNFB 5mM
5.5	4.51	5.06	5.55	6.0	6.51
6.0	4.05	4.54	5.03	5.56	6.06
6.5	3.52	4.05	4.54	5.07	5.57
7.0	3.06	3.53	4.02	4.51	4.52
7.5	2.53	3.07	3.57	3.55	4.05
8.0	2.07	2.02	2.51	3.02	3.98
8.5	1.03	1.58	2.06	2.54	3.73
9.5	0.52	1.01	1.58	2.03	3.54

#### 4.5 Biological engineering (involving artificial intelligence with deep learning in the light of new algorithms in data science) in protein biochemistry confirming future of novel proteins for pharmaceutical industries

The pCa and ROS protection efficiencies of pCa (at high sensitive sites) and DNFB (at low sensitive sites) for *spasmins*, *batonnets* and *ryanodine receptors* (ryr)<sup>38</sup> of spasmoneme were confined in SSrRNA genomic sequences of C+G<sup>39</sup> including molecular symmetric organizations in the form of stereotypic-hindrances interactions<sup>40</sup> including bioenergetics under nuclear analytical evidences consistencies as per parameters of Lennard-Jones and Lazaridis-Karplus, statistical interpolation of hydrogen and disulfide bonding potentials, amino acids compatibility complexes in non-canonical orders of Rosetta, SPRITE and ASSAM for side-chain 3D-motifs and domains preservation/resistance against mutational tendencies of known species.<sup>41, 42, 43</sup> Thus on the basis of results and discussions along with literature cited information is the prime source of information for the next level research extension which can be extended in relation with biomedical support performances at more absolute level in the light of ProFunc/JESS,<sup>44</sup> GIRAF,<sup>45</sup> PRINTS,<sup>46</sup> SPASM & RIGER,<sup>47</sup> SuMo,<sup>48</sup> RASMOT-3D PRO,<sup>49</sup> SA-Mot<sup>50</sup> and ASSAM & SPRITE,<sup>51, 52</sup> DALI,<sup>53</sup> CC RXP<sup>54</sup> and Metapocket 2.0<sup>55</sup> are very important tools for vectorial representations of structural and functional genomics in relation with pCa & ROS differential stress conditions induced *spasmins*, *batonnets* and other resembling and unidentified proteins' frequencies and

durations based protective resistance as in *Vorticella* stalks in the light of molecular therapeutics in most of the Epidemics and Pandemics incoming future situations and thus boosting resistance against ROS based biochemical resistant operations in the form of protein complexities. Thus on the basis of structural and functional efficiencies of biochemical perturbations of *Vorticella* stalks which is being determined on the basis of rRNA based amino acid sequences structures symmetric organizations of *spasmins* and *batonnets* provides hydrophilic and hydrophobic patches of adjacent motifs and their functional orientations to provoke protein-protein interactions in right manner in the form of very strong resistance-recognition as frequencies and durations against ROS production in respect of functional motifs and domain functional feasibilities which can be frequently utilized for energy compensation in terms of thermodynamic energy conservation<sup>56</sup> in conjecture with *patho-physiological* regulation control system under hindrances is mostly clear in the form of given biometric numerical data illustrations (figure - 5) for advanced and novel study formats on the basis of their vectorial operations for new equation dynamics as the case with MATLAB<sup>57</sup> when it is correlated with Raman's spectroscopic investigations for absolute illustration regarding cell motility and contractility of different species and sub-species with higher orders of novel proteins characterizations revealing ROS resistance properties in the form of three dimensional optical symmetrical orientations involving nuclear magnetic resonance (NMR), X-ray crystallographic representations and the molecular-docking experiments.<sup>58, 59, 60</sup>



**Fig – 5: Physicochemical characterizations of *Vorticella* stalk contraction illustration in the light of pCa (in red colour) and DNFB (green colour) dependent concentration gradients from 1 mM to 5 mM in second order of reaction kinetics in the reference of Nernst's, Fenton and Heber-weiss reactivation illustrations acknowledged in the reference of spasmins and batonnets polymeric proteins folding in molten-globular conformational modifications as per ROS and DNP productivities from 5 to 12 pKa value depiction supported by second law of thermodynamics where protonation and deprotonation were in moles per liter, force was in millidyne, power as in ergs/s/g and ΔG as per in kilocalorie/mole reflection.**

#### 4.6 Bio-electro-chemical integration towards the model generation revealing the importance of in-depth scenario in data-science for advanced applications

In association with biochemical foundations for *Vorticella* stalk contractility, quantum/wave mechanics is the modern perspectives where transverse waves on an elastic string in a linear path, where wave is potentialized for threshold power delivery being delivered as fractional wave potentials in Fourier series.<sup>61</sup> Where in their natural/artificial habitats, specimens were kept in aqueous solutions of versatile biochemical solution gradients where biochemical mechano-chemical kinetics were performed as per the laws of quantum chemistry and quantum physics including hydrodynamics as per Navier-Stokes' formula<sup>62</sup> as in *endo-lymphatic* vessels and other cylindrical biodynamic vessels/enclosed compartments as spasmoneme. This description indicated the biomedical importance of wave mechanics in controlling erupted physiological mechanics in respect of new micro-device formulations. Proteins confined in the stalk works on the wave-mechanics as per electro-osmotic flow and regulation systems at the level of bioenergetics investment conservation principles from the point of threshold potential generation to the end point where the wave potential ends at the distal end in three-dimensional perspectives of wave coordinates along the paths of x, y and z in this micro-environmental space system working on the potentials of  $\text{Ca}^{++}/\text{H}^{+}$  against external pCa/DNFB loading conditions (specially time-dependent performance) as discharge/loading potentials in 3D stalk rotational formats regarding signal transductions as per ohm's law in either alpha helical/beta pleated sheets of protein polymers. Where, the immersive breakthrough is indicating that medicinal values of *spasmins/batonnets-isotypes* are being kept in their structural & functional mathematical annotation formats on the basis of primary level amino acids arrangement specifications governing hydrophobic-hydrophilic patches of specified proteins.

#### 4.7 Sequence based protein folding dynamics is full of artificial intelligence for deciphering higher levels of conformational representations for 3D image production, analysis and the processing/mining at very low resolution

The sequence similarities among *spasmins*, *centrins* and *calmodulins* perhaps more than 70% at the higher level of organizations in respect of *triose-phosphate-isomerase*, where the backbone of an alpha-helix is twisted by an equal amount of alpha-carbon with a phi-angle approximation about - 57 degree and a psi angle approximation upto - 47 degrees where the complete turn of alpha-helix is approximately 3.6 amino-acyl residues with a pitch of 0.54 nm in terms of Bragg's equation as  $2d\sin\theta = n\lambda$  where d is the distance between atomic layers in a crystal,  $\lambda$  is the wavelength of the incident X-ray beam,  $\theta$  is the angle of incident and n is the integer by involving principles of Pythagoras theorem as in Ramachandran plot depiction, where the R-groups of each amino-acyl residue in an alpha ( $\alpha$ )-helix face outward and the protein contain only  $\text{L-}$  amino acids for which, a right handed  $\alpha$  - helix by far, hence is more suitable, hence only  $\alpha$  - helix occurs in nature as in *spasmin*, *centrin*, or *calmodulin* in cylindrical organizations.<sup>63</sup> Here in these structural organizations, the stability of  $\alpha$  - helix is confirmed by hydrogen bonds between oxygen of carbonyl and hydrogen

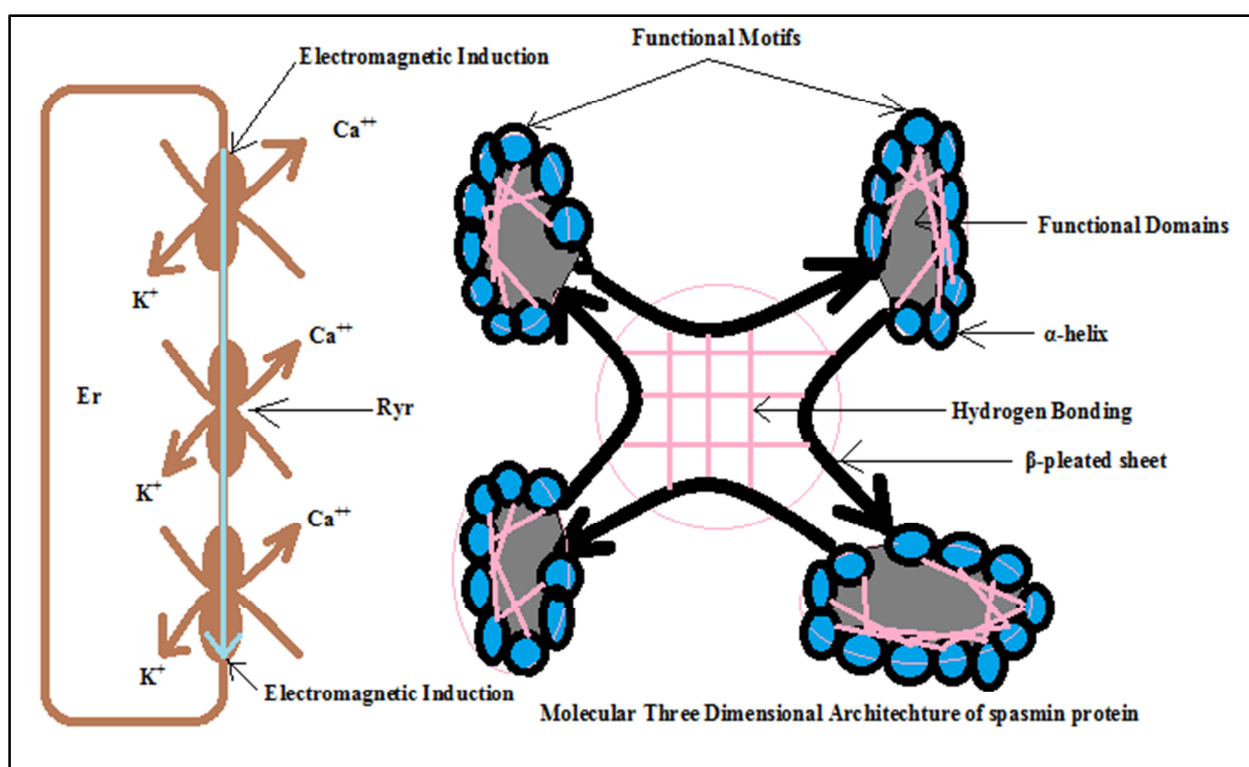
or peptide-bonded-nitrogen of the fourth residue. As per the number of hydrogen bonds towards the centre, it was supplemented by van der Waal forces of attraction for structural stabilities, being strongly supported by thermodynamic driven forces. In this thermodynamic support system, nitrogen of *proline* taking part in peptide bond formation lacks hydrogen and thus hydrogen bond is confirmed to accommodate  $\alpha$ -helix turn conformity. In these structural organizations, when *proline* is shifted, a bend is produced as per the case with glycine, but still there are great differences in the patterns of folding as per taken by *proline* and *glycine* to establish end-to-end inscriptions with beta ( $\beta$ )-pleated sheet organizations.<sup>64</sup> In  $\alpha$ -helix structural organizations, hydrophobic R-groups were on one side of the axis, whereas hydrophilic patches where on another side. Such *amphipathic* helix organizations established interactions of polar and *apolar* regions of hydrophobic interiorities with aqueous solutions, where clusters of *amphipathic* helix permits the *ryr*-receptors to open the gets for  $\text{Ca}^{++}/\text{K}^{+}$  exchange across the luminal compartments of ER and separated by extra-luminal matrices of *sacculles* inside the spasmoneme of the stalk.<sup>65</sup> Here in these cases, half amino acid residues were taken in  $\alpha$ -helical and  $\beta$ -sheets organizations as in turns, bends & loops formation whereas remaining 50% were involved in structural stabilizations. In these cases, turns and bends were referred as the milestones of secondary conformational organizations by involving *amino-acyl* residue as a first residue in hydrogen bonding with fourth residue at 180° per turn, where *proline/glycine* is often located where  $\beta$ -sheet takes a turn.

#### 4.8 Model given in this paper (figure - 6) involving biochemical networking in terms of signal transduction

Here, domains forming bridge connecting substrate for functional annotations as pCa/H<sup>+</sup> along the lengths of charged amino acid residues participated in enzyme-catalyzed reactions as amino-acyl in contraction-extension cyclic processes in respect of pCa/DNFB binding affinities of *spasmins* & *batonnets* with respective domains participating in loop, turn and bend formation<sup>66</sup> (figure - 6). These loops, turns and bends at the levels of biochemical catalytic reactions on the basis of sequential arrangements as per R-groups of hydrophobic and hydrophilic patches of sequences confirmed their behaviours as per the medium in which they were placed as either acidic, alkaline, neutral, pCa & DNFB concentrations through undergoing *acetylation*, *acylation*, *methylation*, *phosphorylation*, *hydroxylation*, *carboxylation*, *O & N-linked glycosylation*, *myristoylation*, *palmitoylation*, *fernesylation*, *biotinylation*, *ribosylation* of the concerned residues of *spasmins* as well as *batonnets* to accelerate the folding dynamics in their 3D rotational orientations in terms of crystallographic image formation as per the molecular organizations of either  $\alpha$ -helices &  $\beta$ -pleated sheets of concerned proteins reflecting stereo-chemical molecular orientations in their given/confirmed spaces of orientations (*cis/trans orientations*) under the hindrances of biophysical laws of physics as well as physical chemistry under the regulations of *mathematical annotations* in a very complex organizations in a liquid medium of cytoplasmic matrix of viscous drag force resistance as per tangential performances of molecular coordinates along the axis of x, y and z axes in a confirmed *micro-nitche* space in micro-fluidic aqueous medium of fixed



electro-chemical characterizations for better and absolute experiences.



**Fig – 6: A composite molecular model hypothesis for *spasmin*'s protein structural organization with defined functional motifs and domains where two bio-molecular arms stands for each pCa incorporation for initial signal transduction involved in *Vorticella* stalk contraction dynamics in relation with electrophysiological exchange processes for the purpose of force and power generation in terms of energy conservative proto osmotic repeat performances for future benefits.**

## 5. CONCLUSION

Thus on the basis of results and discussions we can established biodynamic correlations between structure and function of reference proteins' byproducts at the fundamental level which can be upgraded in the light of protein engineering technology based on genomic hindrances for future utilizations to combat ROS based *torsion-detorsion* structural modifications tolerance compatibility complexes at the level of *patho-physiological* demonstrations and bio-molecular inductive forces and power production dependent metabolic disorders by DNP production management hindrances. Thus this article provide strength for the higher order information sicker and scientists to mold their ideology towards artificial protein engineering productions in the light of *microgenic-proteomic-studies* to support motility and contractility based metabolic disorder corrections in the light of modern bioinformatics' tools and techniques in current scenario for most of the cases including cell motility and contractility in respect of human health securities, food processing concerned with blue revolution and the beauty of face and mind, all are hidden under bioinformatics deciphering new information to boost day today scientific

investigations in the light of metabolism disorders which can be used to treat correlative pandemic/epidemic diseases on the basis of novel proteins characterizations such as *spasmins*, *batonnets* and other mysterious proteins occupied in microscopic natural environments.

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## 7. AUTHORS CONTRIBUTION STATEMENT

This work was done by the author Dr. Amit Kumar Verma during the period of 2010-2014 in P. G. Department of Zoology, Jai Prakash University, Chapra, Bihar, India, and it is the part of Ph. D. Thesis.

## 8. CONFLICT OF INTEREST

Conflict of interest declared by author is none for this article.

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