ONOMY Science

Scientific Journal of Material Science & Engineering

Research Article Open Access

Enzyme Functionality: Zn2+-Chelating Peptide and Reaction of Zinc Enzymes

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Citation: Enzyme Functionality: Zn2+-Chelating Peptide and Reaction of Zinc Enzymes. Sci J Mat Sci & Eng. 2019; 1(1): 001-008.

Submitted: 16 May 2019; **Approved:** 22 May 2019; **Published:** 24 May 2019

Abstract

The display of peptide sequences on the surface of bacteria is a technology that offers exciting applications in biotechnology and medical research. Type 1 fimbriae are surface organelles of Escherichia coli which mediate d-mannose-sensitive binding to different host surfaces by virtue of the FimH adhesin. FimH is a component of the fimbrial organelle that can accommodate and display a diverse range of peptide sequences on the E. coli cell surface. In this study we have constructed a random peptide library in FimH. The library, consisting of ~40 million individual clones, was screened for peptide sequences that conferred on recombinant cells the ability to bind Zn2+. By serial selection, sequences that exhibited various degrees of binding affinity and specificity toward Zn2+ were enriched. None of the isolated sequences showed similarity to known Zn2+-binding proteins, indicating that completely novel Zn2+-binding peptide sequences had been isolated. By changing the protein scaffold system, we demonstrated that the Zn2+-binding seems to be uniquely mediated by the peptide insert and to be independent of the sequence of the carrier protein. These findings might be applied in the design of biomatrices for bioremediation purposes or in the development of sensors for detection of heavy metals.

INTRODUCTION

The potential threat of heavy-metal and radionuclide pollution for ecosystems and public health has led to an increased focus on the development of systems for their sequestration and removal from soil, sediment, and wastewater. So far, decontamination techniques have been based mostly on traditional physiochemical methods, but in recent years interest has also centered on the application of biotechnology to efficient waste treatment. To this end, a number of biological remediation systems have been established in bacteria, algae, fungi and plants (5, 11, 17, 26).

Expression of heterologous peptides in naturally occurring surface proteins has become a powerful tool in generating microorganisms with binding affinity toward specific target molecules. This technique has been employed in the development of recombinant live vaccines, reagents for diagnostics, antibody production, screening of peptide libraries, and design of microbial biocatalysts and has recently constituted an attractive approach to development of bacterial bioadsorbents for heavy-metal removal purposes (2, 9, 10, 15).

Random peptide library expression is a highly versatile technology. Systems in which such libraries are expressed in connection with a surface protein scaffold allow the screening of a huge number of peptides (~108) from which binders to a particular molecular target can be isolated by various panning techniques (6).

A well-characterized scaffold system for display of heterologous peptides is based on type 1 fimbriae. These are hair-like surface organelles present on most members of the Enterobacteriaceae. Type 1 fimbriae are found in up to 500 copies on the cell; they are heteropolymers, and each fimbria consists of about 1,000 copies of the major structural subunit, FimA. The d-mannose-specific FimH adhesin, located on the tip and perhaps also intercalated along the organelle, is also a structural component. By site-directed mutagenesis, we have previously identified permissive sites in FimH that allow the insertion and surface display of heterologous se

quences without altering the overall structure and function of FimH (14, 21). Such sites have been used for display of vaccine-relevant epitopes (14). Recently, we have successfully used the FimH protein as a molecular scaffold for the display of random peptide libraries (7, 19, 20). In this paper we report the identification of novel Zn2+-binding peptides selected from a FimH-displayed random peptide library. Our results indicate that the zinc binding can be a unique property of the displayed peptide and independent of the protein scaffold.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. In this study we used the E. coli K-12 strain S1918 (F′ lacIq ΔmalB101 endA hsdR17 supE44 thiI relA1 gyr-96 ΔfimB-H::kan) (3). Cells were grown in Luria-Bertani medium supplemented with the appropriate antibiotics. Our FimH display system consists of two plasmids, the FimH expression vector pLPA30 and an auxiliary plasmid pPKL115. Plasmid pLPA30 is a pUC18 derivative containing the fimH gene downstream of the lac promoter. A BglII linker, located in a position corresponding to amino acid 225 (14), was used for integration of the random library. Plasmid pPKL115 is a pACYC184 derivative containing the whole fim gene cluster with a translational stop linker inserted in the fimH gene (14).

DNA techniques. Plasmid DNA was isolated using the QIAprep Spin Plasmid kit (Qiagen). Restriction endonucleases were used as specified by the manufacturer (Biolabs or Pharmacia). PCR amplifications to monitor the size and distribution of the random library were performed as previously described (24). The oligonucleotide primers used in these reactions were P1 (5′-CCTGCACAGGGCGTCGGCGTAC) and P2 (5′-GGAATAATCGTACCGTTGCG). The nucleotide sequences of inserts conferring on cells the ability to bind to metal oxides were determined by the dideoxynucleotide chain termination method (18).

Construction of the random peptide library. Construction of the random library was performed essentially as described by Brown (3). Briefly, a template oligonucleotide containing the sequence 5′-GGACGCAGATCT(VNN)9AGATCTAGCACCAGT-3′ (where N indicates an equimolar mixture of all four nucleotides and V indicates an equimolar mixture of A, C and G) was chemically synthesized. A primer oligonucleotide, 5′-ACTGGTGCTAGATCT-3′, was hybridized to the template oligonucleotide and extended with the Klenow fragment of DNA polymerase I. The double-stranded oligonucleotide was purified by phenol-chloroform extraction and digested with BglII to release an internal 33-bp fragment. This was purified by electrophoresis through a 12% polyacrylamide gel in Tris-borate-EDTA (TBE) and eluted into a buffer containing 10 mM Tris-HCl (pH 8.0), 2 mM EDTA, and 0.15 M NaCl. The eluate was filtered through a 0.22-μm-pore-size Qiagen filter, concentrated by ethanol precipitation, and redissolved in a buffer containing 10 mM Tris-HCl (pH 8.0), 1 mM EDTA, and 0.1 M NaCl. The redissolved 33-bp BglII fragment was ligated at various ratios to BglII-digested pLPA30. The ligation products were precipitated with ethanol and electroporated into S1918(pPKL115).

The diversity of the library was calculated to be 4 × 107 individual clones based on extrapolation from the numbers of transformants obtained in small-scale platings. The transformation mixture was made up to 10 ml and grown for approximately seven generations (4 × 109 cells). Aliquots (1 ml) were frozen at −80°C in 25% (vol/vol) glycerol. Each 1-ml aliquot contained approximately 4 × 108 cells, which represented 10 times the library diversity. Random screening of clones by PCR revealed a predominance of one to three 33-bp oligonucleotide inserts; sequencing of the inserts from randomly selected clones revealed G+C contents ranging from 30 to 70%.

Enrichment procedure. Bacterial cells were bound to zinc ions by use of stripped Ni2+-nitrilotriacetic acid (NTA) solid matrix (Qiagen) recoated with Zn2+ by a standard method. The enrichment procedure for identifying Zn2+-binding clones from the random library was as follows. Mid-exponential-phase cultures were diluted into M63 salts (13) containing 20 mM methyl α-d-mannopyranoside and 50% (vol/vol) Percoll (Pharmacia). The methyl α-d-mannopyranoside was added to block the natural receptor-binding domain of the FimH adhesin. The use of Percoll permitted the formation of a density gradient on centrifugation, which resulted in a distinct band due to the Zn2+-NTA resin, and specific separation of any adherent bacteria from nonadherent bacteria. Under these conditions, bacteria expressing wild-type FimH proteins as components of type 1 fimbriae did not coseparate with the Zn2+-NTA resin. The resin and bacteria expressing the random peptide library within FimH were mixed and allowed to adhere at room temperature with gentle agitation. Centrifugation was then performed, and the resin and any adhering bacteria were recovered and inoculated into Luria-Bertani medium containing appropriate antibiotics. After overnight incubation, exponentially growing cultures were established and the enrichment procedure was repeated. Following each cycle of enrichment, aliquots of the populations were stored at −80°C. Plasmid DNA was prepared from each aliquot and used in PCR to monitor the size distribution of the inserts in the population as previously described (19).

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Binding assay and quantification. Mid-exponential-phase cultures standardized on the basis of their optical density at 550 nm (OD550) were washed and resuspended in M63 salts containing 20 mM methyl α-d-mannopyranoside. Samples were incubated at room temperature for 15 min with gentle agitation before the addition of Zn2+-NTA agarose beads. After a 15-min incubation with gentle agitation, the beads were examined by phase-contrast microscopy (Carl Zeiss Axioplan microscope) and digital images were captured with a 12-bit cooled slow-scan charge-coupled device camera (KAF 1400 chip; Photometrics, Tucson, Ariz.) controlled by PMIS software (Photometrics).

The ability of individual clones to bind to Zn2+ was measured by counting cells attached to a selection of randomly chosen Zn2+-NTA beads and correlating the number of adhering cells to the bead size. The same procedure was used for quantification of cells binding to Ni2+-NTA and Cu2+-NTA beads.

Agglutination of yeast cells. The capacity of bacteria to express a d-mannose-binding phenotype was assayed by their ability to agglutinate yeast cells (Saccharomyces cerevisiae) on glass slides. Aliquots of washed bacterial suspensions at an OD550 of 1.0 and 10% yeast cells were mixed, and the time until agglutination occurred was measured.

Insertion of a CTB loop in fimH. Two oligonucleotides, oligonucleotide KK12 (5′-GATCTGTTGAAGTTCCGGGATCCCAGCATATCGATAGTCAGAAA AAAGCTA-3′) and oligonucleotide KK13 (5′-GATCTAGCTTTTTTCTGACTATCGATATGCTGGGATCCCGGAACTTCAACA-3′) encoding amino acids 50 to 64 of cholera toxin B chain (CTB), were designed so that they contained an internal BamHI site at amino acid position 54 and were flanked by BglII overhangs. These oligonucleotides were annealed, phosphorylated, and ligated into pLPA30 digested with BglII. The resultant plasmid (pKKJ16) was checked by BamHI digestion and sequencing. Plasmid pKKJ16 (containing the loop of CTB in fimH) was transformed into S1918(pPKL115).

Engineering a Zn2+-binding peptide into the CTB3 loop in FimH. The Zn2+-binding sequence of pKKJ106 was amplified by PCR using primers KK77 (5′-GCCCGGATCCGAAAGCAGGGTCGACC-3′)and KK78 (5′-GCCCGGATCCTTGGTGATGACGCTCTG-3′) containing BamHI overhangs. The PCR product was digested with BamHI and ligated into pKKJ16 digested with BamHI. The resultant plasmid (pKKJ145) was checked by sequencing and transformed into S1918(pPKL115).

Fimbria purification. OD550-standardized overnight cultures were harvested by centrifugation and washed with phosphate-buffered saline (PBS). Cells were resuspended in PBS, and fimbriae were detached from the cell surface by blending. The cell debris was removed by centrifugation, and the fimbriae in the supernatant were precipitated with acetone. The purified fimbriae were dried and resuspended in PBS (8).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western immunoblotting. Purified fimbriae were treated with diluted HCl (pH = 2) and separated on 15% polyacrylamide gels by sodium dodecyl sulfate-polyacrylamide gel electrophoresis by using standard procedures (16). The gels were transferred to polyvinylidene difluoride microporous membrane filters using a semidry blotting apparatus. The membranes were blocked with 0.5% Tween 20 and incubated with anti-FimH (truncated) serum followed by horseradish peroxidase-conjugated anti-rabbit serum.

RESULTS

Library construction in FimH. A random peptide library based on oligonucleotides 33 bp in length with BglII overhangs was constructed for display in the type 1 fimbria adhesin FimH (Fig. (Fig.1).1). To this end, we used a vector (pLPA30) containing the fimH gene with a BglII linker inserted at codon position 225 and under the transcriptional control of the lac promoter (14). Insertions in this position have previously been shown to permit the expression of heterologous sequences without affecting the properties of FimH. The inserted double-stranded oligonucleotides consisted of nine random codons flanked by BglII restriction sites (encoding Arg-Ser). Due to the presence of BglII overhangs, various numbers of double-stranded oligonucleotides were inserted in fimH, further adding to the complexity of the library. To express FimH variants as constituents of fimbriae, an auxiliary plasmid (pKKL115), containing all fim genes except fimH, was used for transcomplementation of the fimH-containing plasmid. Expression from the binary plasmid system led to display of chimeric FimH in the context of fully functional fimbriae.

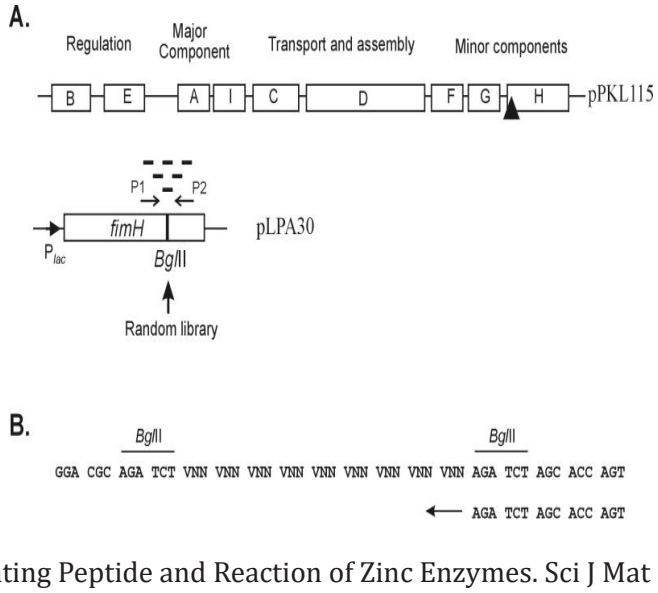

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FIG. 1: Overview of random peptide display in type 1 fimbriae. (A) The binary plasmid systems used in heterologous display by FimH. Plasmid pPKL115 contains the entire fim gene cluster with a translational stop linker inserted in the fimH gene (indicated by the solid triangle). The FimH expression vector pLPA30 is shown, along with the BglII insertion site at amino acid 225 and the two primers (P1 and P2) used to monitor the size and distribution of the random library. (B) Genetic structure of the random library inserted into fimH. The two oligonucleotides were annealed and extended with the Klenow fragment of DNA polymerase I, and the product was purified after digestion with BglII. N indicates an equimolar mixture of all nucleotides, and V indicates an equimolar mixture of A, C, and G. The use of a VNN coding system prevents the introduction of functional stop codons in an amber-suppressing host.

Selection and identification of Zn2+-binding sequences. Cells able to adhere to Zn2+ were isolated from the FimH-displayed random library after repetitive rounds of selection. The cells were allowed to bind to Zn2+-NTA beads, and binding cells were separated from nonbinders by density gradient centrifugation in 50% (vol/vol) Percoll. Bacteria adhering to the Zn2+-NTA beads were recovered and transferred to fresh growth medium. The enrichment procedure was repeated, and the insert distribution of the population was monitored by PCR (data not shown). No change in the insert population was observed in a control experiment, in which neither Zn2+-NTA nor Percoll was present during the enrichment procedure. However, a notable change in the insert distribution was observed after three rounds of enrichment with Zn2+-NTA. Cells obtained from the third enrichment cycle were spread onto agar plates, and cultures were established from 20 single colonies. The ability of cells expressing the enriched peptides to adhere to Zn2+-NTA was examined by phase-contrast microscopy (Fig. (Fig.2).2). Of the 20 clones, 15 displayed a Zn2+-binding phenotype. To ensure that the observed binding phenotype was indeed FimH based, each of the fimH-encoding plasmids was isolated and retransformed into S1918(pPKL115). The new recombinant clones displayed the same binding phenotype as the original isolates, indicating that the binding phenotype was indeed plasmid encoded. Furthermore, the agglutination titers of these cells were similar to that of a control strain expressing wild-type FimH, indicating that the presence of the inserts had not significantly altered the amount of surface-displayed FimH.

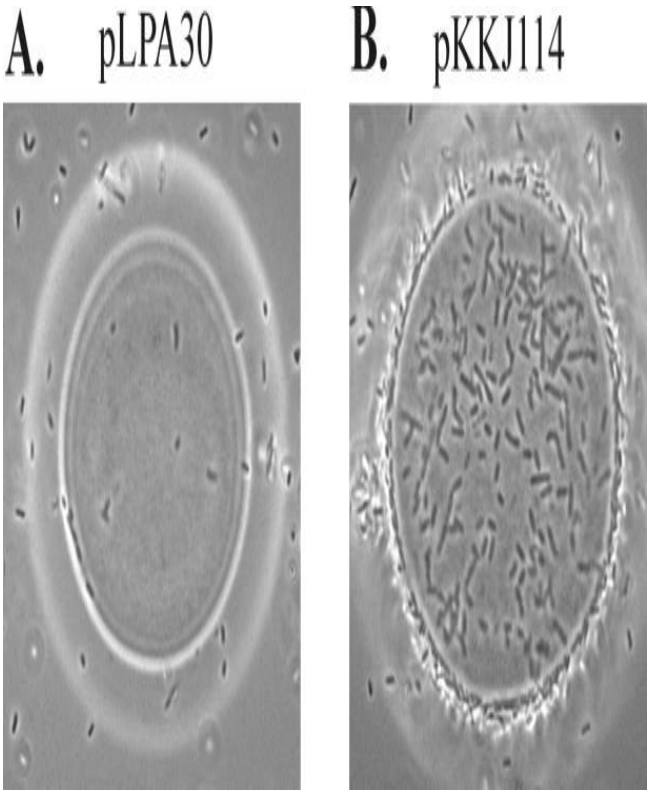

FIG. 2: Phase-contrast microscopy showing adherence to Zn2+-NTA beads by S1918(pPKL115) cells containing plasmid pLPA30 (wild-type fimH) (A) or plasmid pKKJ114 (random library clone isolated after selection for adherence to Zn2+-NTA beads) (B).

Most of the isolated sequences contained one or more histidine residues, as expected given the important role played by this amino acid in Zn2+ binding. It is a well-established fact that histidine is able to chelate divalent metal ions, as seen in a number of proteins with zinc finger motifs and metallothioneins (25). However, one sequence (pKKJ113) devoid of histidines was also identified from the library, showing that histidine is not an absolute requirement for binding to Zn2+. Indeed, cells expressing the peptide sequence of plasmid pKKJ113 mediated stronger Zn2+ binding than did cells expressing peptides containing multiple histidine residues. Furthermore, plasmid pKKJ113 displayed a very high degree of binding specificity toward Zn2+ compared to its specificity toward Ni2+. Previously, Barbas et al. (1) identified a number of Zn2+-binding peptides from a phage-displayed semisynthetic combinatorial antibody library. We did not observe any similarities between our Zn2+-binding sequences and those identified by Barbas et al. (1). This might be due to the genetic structure of the libraries and the different selection and enrichment procedures employed.

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REFERENCES

1. Early Alzheimer's Disease: Patterns of Functional MRI Activation-The Neural Substrates of Semantic Memory Deficits. Am J Bra Dis and Tum. 2018; 1(1): 001-010.
2. H Chahal, S W D Souza, A J Barson and P Slater. How to develop human brain using magnesium of N-methyl-D-aspartate receptors, Am J Bra Dis and Tum. 2018; 1(1): 001-005.
3. F S LaBella, et al.Concepts and correlations related to general anaesthesia and cytochrome P450 oxygenases. Am J Anest and Pai med. 2018; 1(1): 01-05.
4. Hazim J Safi, et al. The long term method with the elephant trunk for the repair of aortic aneurysms. Am J Anest and Pai med. 2018; 1(1): 001-008.
5. Yoshitaka Fujii, et al. Diaphragmatic Fatigue is treated with Inhaled Aminophylline Therapy in an Experimental Canine procedure. Am J Anest and Pai med. 2018; 1(1): 001-003.
6. O Demirkiran, et al. Complications in patients with Crush syndrome after the Marmara earthquake. Am J Anest and Pai med. 2018; 1(1): 001-005.
7. Qi Wei, et al. Laparoscopic choledochotomy after Biliary drainage: Study. Am J Anest and Pai med. 2018; 1(1): 001-007.
8. Mark Palazzo, et al. Unilateral Babinski/Plantar Reflex - Acute Inflammatory Demyelinating Polyneuropathy. Am J Anest and Pai med. 2018; 2(1): 01-02.
9. Hakan Alfredson, et al. Achilles and patellar tendon operations performed in local anestesia, Am J Anest and Pai med. 2018; 1(1): 001-002.
10. Naemeh Nikvarz, et al. Evaluation The Analgesic Effect of Duloxtine Drug in Burn Pationts. Am J Anest and Pai med. 2019; 2(1): 01-07.
11. Chuandong Zheng, et al. Intravascular Plaque: Cause for Radial Arterial Catheterization Failure. Am J Anest and Pai med. 2019; 2(1): 01-05.
12. Laura Tyler Perryman, et al. Wireless Dorsal Root Ganglion Stimulation: An Introduction and Early Experience with the New Approach for Chronic Pain Management. Am J Anest and Pai med. 2019; 2(1): 01-04.
13. Lazraq Mohamed, et al. Pediatric Pre-Anesthesia Consultation: What are Parents Expectations?. Am J Anest and Pai med. 2019; 2(1): 01-02.
14. Alaa Ali M. Elzohry, et al. Safety and Efficacy of Intraperitoneal Irrigation of Levo-Bupivacaine plus Morphine in Patients Undergoing Major Abdominal Cancer Surgeries. Am J Anest and Pai med. 2019; 2(1): 01- 07.
15. Yıldız K, et al.Comparison between Anesthesia Methods In Orthopaedics Initiatives of Upper Extremity. Am J Anest and Pai med. 2019; 2(2): 01-03.
16. Jianming Liu, et al. The Analgesic Effects Nalbuphine Hydrochloride Combined With Sufentanil for Patients after Thoracoscopic Lobectomy. Am J Anest and Pai med. 2019; 2(2): 01-03.
17. Fudong Shi, et al. The Patient Controlled Intravenous Analgesia of Dezocine on the Elderly Patients After Orthopedic Surgery. Am J Anest and Pai med. 2019; 2(1): 01-04.
18. GE Meglia, et al. Investigation in blood Leukocytes and Neutrophils in Periparturient Dairy Cow. Sci J of Ani and Vet Sci. 2018; 1(1): 001-009.
19. G E Duhamel, et al.DNA Sequence Analysis of an Immunogenic Glucose-Galactose Mglb. Sci J of Ani and Vet Sci. 2018; 1(1): 001-009.
20. David G. White, et al. Chloramphenicol and Florfenicol Resistance in Escherichia Coli of Characterization . Sci J of Ani and Vet Sci. 2018; 1(1): 001-006.
21. N B Alhaji, et al. Anophthalmia and Choanal Atresia In Two Months Old Kid. Sci J of Ani and Vet Sci. 2018; 1(1): 001-004.
22. Christopher W Olsen, et al.Isolation and Characterization of H4N6 Avian and Influenza Viruses. Sci J of Ani and Vet Sci. 2018; 1(1): 001-0025.
23. Teresa Lopez-Arteaga, et al. Apathy as a Psychiatric Manifestation of Meningioma. Am J Bra Dis and Tum. 2018; 1(1): 001-004.
24. David R Murdoch, et al. The Use of Brain Natriuretic Peptide- Whole Blood can be Measured, Am J Bra Dis and Tum. 2018; 1(1): 001-003.
25. Stefan Brocke, et al. Antibodies to Integrin α4 and CD44, but not CD62L, Prevent CNS Inflammation and Experimental Encephalomyelitis by Blocking Secondary Leukocyte Recruitment. Am J Bra Dis and Tum. 2018; 1(1): 001-006.
26. Andrew J Saykin, et al. Early Alzheimer's Disease: Patterns of Functional MRI Activation-The Neural Substrates of Semantic Memory Deficits. Am J Bra Dis and Tum. 2018; 1(1): 001-010.
27. P Slater, et al.How to develop human brain using magnesium of N-methyl-D-aspartate receptors, Am J Bra Dis and Tum. 2018; 1(1): 001-005.
28. Clyde W Hodge, et al. The Paraventricular Nucleus Interactively Modulate Ethanol Consumption -Norepinephrine and Serotonin Receptors, Am J Bra Dis and Tum. 2018; 1(1): 001-005.
29. Paulo C Carvalho, et al. Bioinformatics grid application in simple - Squid. Sci J Biome and Biost. 2018; 1(1): 001-004.
30. Mahmoud A E Abdelrahman, et al. On The New Exact Solutions for the Nonlinear Models Arising In Plasma Physics. Sci J Biome and Biost. 2018; 1(1): 001-004.
31. Weicheng Shen, et al. Based on Personal Identification- Automated Biometrics. Sci J Biome and Biost. 2018; 1(1): 001-002.
32. V Prasathkumar, et al.Fingerprint Biometric System - Using of Personal Authentication. Sci J Biome and Biost. 2018; 1(1): 001-003.
33. SavitaChoudhary, et al. Software Development Environment : Design of Biometric Based Transaction System. Sci J Biome and Biost . 2018; 1(1): 001-003.
34. D J Lawrence, et al. Measuring the effectiveness in reliability and validity of a visual function outcomes instrument in cataract surgery. Sci J Biome and Biost. 2018; 1(1): 001-004.
35. Z Suvakovic, et al. Evaluation of early detection of gastric cancer requries more than gastroscopy. Anna of Can Ther and Phar. 2018; 1(1): 05.
36. Ho GY, et al. Informing and involving personalised computer based data for cancer patients. Anna of Can Ther and Phar. 2018; 1(1): 001-005.
37. Ray Jones, et al. Prostate Cancer Risk is associated with Polymorphism of Insulin gene. Anna of Can Ther and Phar. 2018; 1(1): 001-005.
38. : Jean-Pierre J. Issa, et al. Role of DNA Methylation in Tumor Suppressor Gene Silencing in Colorectal Cancer. Anna of Can Ther and Phar. 2018; 1(1): 001-008.
39. Jules J Berman, et al. Histological classification of tumour and molecular analysis meets Aristotle. Anna of Can Ther and Phar. 2018; 1(1): 001-005.
40. Kafil Akhtar, et al. Tuberculosis of the Tongue with Coexistent Squamous Cell Carcinoma: An Interesting Case Presentation, Anna of Can Ther and Phar. 2018; 1(1): 001-002.
41. Serafin Morales Murillo, et al. Vitamin D as A Prognostic Factor in Triple Negative Breast Cancer. Anna of Can Ther and Phar. 2019; 2(1): 01-08.
42. Ahmet Fuat, et al. A Qualitative Study of Accurate Diagnosis and Effective Management of Heart Failure in Primary Care. Am J of Card and Cardiovas Disc. 2018; 1(1): 01-05.
43. Jesús Millán Núñez-Cortés, et al. Prescription Habits for Statins in Patients with Impaired Glucose Metabolism. Results of a program with Focus Groups to Assess the Selection Criteria. Am J of Card and Cardiovas Disc. 2019; 1(1): 01-04.
44. G D Kolovou, et al. Evaluation of Postprandial hyper

Cite this article: Enzyme Functionality: Zn2+-Chelating Peptide and Reaction of Zinc Enzymes. Sci J Mat Sci & Eng. 2019; 1(1): 001-008.

triglyceridaemia in patients withTangier disease. Am J of Card and Cardiovas Disc. 2018; 1(1): 01-04.
45. Brian O rourke, et al. Determination of The Mitochondrial Redox Waves and Subcellular Metabolic Transients in Heart Cells. Am J of Card and Cardiovas Disc. 2018; 1(1): 01-04.
46. Shuixiang Yang, et al. Radiofrequency Ablation Treating Atrial Fibrillation Can Reverse the Changes of Mirnas Regulating Ion Channel Proteins. Am J of Card and Cardiovas Disc. 2018; 1(1): 01-08.
47. Hadi abdulsalam Abo Aljadayel, et al. Penetrating War Cardiac and Great Vessels Injury, Surgical Outcome Analysis in 24 Patients. Am J of Card and Cardiovas Disc. 2018; 1(2): 01-05.
48. Hatice Yorulmaz, et al. Assessment of the Death Anxiety and Death Depression Levels of Cardiac Patients. Am J of Card and Cardiovas Disc. 2019; 2(1): 01-06.
49. Camara Abdoulaye, et al. Cardiomyopathie Du Peripartum Compliquee D'accident Vasculaire Cerebral Cas D'une Guinéenne De 19ans : Cas Clinique. Am J of Card and Cardiovas Disc. 2019; (1): 01-03.
50. Sergio F. Estrada-Orihuela, et al. Lasalocid, Interrupts and Reverses, Within One Minute, The Myocardial Damage Caused By Coronary Anoxia Reperfusion in Rat Heart. Am J of Card and Cardiovas Disc. 2019; (1): 01-05.
51. Jesus Millan Nunez-Cortes, et al. Prescription Habits for Statins in Patients with Impaired Glucose Metabolism. Results of a program with Focus Groups to Assess the Selection Criteria. Am J of Card and Cardiovas Disc. 2019; 1(1): 01-06.
52. Federico Cacciapuoti, et al. The Dilemma of Diastolic Heart Failure. Am J of Card and Cardiovas Disc. 2019; 1(1): 01-03.
53. Elad Boaz, Bowel Ischemia and Vascular Air-Fluid Levels. Anna Cas Rep and Ima Surg. 2018; 1(1): 001-00.
54. Sinisa Franjic, et al. A Patient With A Maxillofacial Problem. Anna Cas Rep and Ima Surg. 2018; 1(1): 001-004.
55. Davidson W, et al. Case Presentation: Hantavirus pulmonary syndrome [HPS]. Anna Cas Rep and Ima Surg. 2018; 1(1): 001-005.
56. Farid ZM, et al. Uropathy Secondary Chronic obstructive to Ureter Inguinal Herniation. Anna Cas Rep and Ima Surg. 2018; 1(1): 001-002.
57. De Letter DJ, et al. Cornual Molar Ectopic Pregnancy Diagnosis and Treatment. Anna Cas Rep and Ima Surg. 2018; 1(1): 001-003.
58. Ameni Touati, et al. Silver Russell Syndrome: Case Reports from North Africa and Review on The Literature. Anna Cas Rep and Ima Surg. 2019; 1(1): 001- 004.
59. Kunst WM, et al. Case Reports and Review of Spontaneous Rupture of Hyperreactive Malarial Splenomegaly [HMS]. Anna Cas Rep and Ima Surg. 2018; 1(1): 001-005.
60. F Hanefeld, et al. A Review of The Literature an Emerging Community Pathogen methicillin-Resistant Staphylococcus. Anna Cas Rep and Ima Surg. 2018; 1(1): 001-0011.
61. Page W Caufield, et al. Evidence for a Discrete Window of Infectivity. Am J Den and Ora Car. 2018; 1(1): 001-006.
62. Robert T Dirksen, et al. Dihydropyridine Receptors and Ryanodine Receptors: Bi-Directional Coupling . Am J Den and Ora Car. 2018; 1(1): 001-009.
63. IJ Jacobs, et al. Cancer and Intraepithelial Neoplasia-Tissue-specific apoptotic effects of the p53 codon 72 polymorphism . Am J Den and Ora Car. 2018; 1(1): 001-003.
64. Iain L C Chapple, et al. Human Immunodeficiency Virus disease in oral health significances. Am J Den and Ora Car. 2018; 1(1): 001-007.
65. H Larjava, et al. Activity of αvβ6 Integrin in Oral Leukoplakia. Am J Den and Ora Car. 2018; 1(1): 001-005.
66. Siddharth Kothari, et al. Effectiveness of Counselling and Home Care Self-Management Strategies in Reducing Masticatory Muscle Pain: A Review. Am J Den and Ora Car. 2019; 2(1): 001-007.
67. Betania Maria Soares, et al. Use of Blue LED and Curcumin for Photosensitization of Candida Albicans. Am J Den and Ora Car. 2019; 2(1): 001-005.
68. Jing Guo, et al. Advances in Methods of Maxillary Transverse Expansion. Am J Den and Ora Car. 2019; 2(1): 01-05.
69. Dario C. Altieri, et al. Cell division by p34cdc2 phosphorylation of survivin- Regulation . Sci J of Der and Ven. 2018; 1(1): 001-005.
70. Axel Trautmann, et al. Eczematous dermatitis: T cell and keratinocyte apoptosis plays a key pathogenetic . Sci J of Der and Ven. 2018; 1(1): 001-007.
71. JD Fine, et al. Epidermolysis bullosa Cardiomyopathy in inherited . Sci J of Der and Ven. 2018; 1(1): 001-004.
72. NE Fusenig, et al. Human Skin Angiogenic Switch Occurs Squamous Cell Carcinomas . Sci J of Der and Ven. 2018; 1(1): 001-007.
73. Tapani Tuomi, et al. Water- Damaged Building and Mycotoxins in Crude Building Materials. Sci J of Der and Ven. 2018; 1(1): 001-005.
74. John S Davies, et al. The Use of Social Media among Doctors Under taking a Post-Graduate Endocrinology Diploma. Sci J Endo and Meta. 2018; 1(1): 001-004.
75. Juan J Gagliardino, et al. By Short-Term Dietary Manipulation: The Endocrine Pancreas Activity of Tyrosine Hydroxylase. Sci J Endo and Meta. 2018; 1(1): 001-005.
76. Colin A. Leech, et al. The Glucose Dependent in Pancreatic β-Cells : Voltage-Independent Calcium Channels Mediate Slow Oscillations of Cytosolic CalciumPancreatic β-Cells. Sci J Endo and Meta. 2018; 1(1): 001-009.
77. Colin A. Leech, et al. The Voltage-Independent Activation of Inward Membrane Currents and Elevation of Intracellular Calcium in HIT-T15 Insulinoma CellsPituitary Adenylate Cyclase-Activating Polypeptide Induces. Sci J Endo and Meta. 2018; 1(1): 001-008.
78. Suhail AR Doi, et al. Making Use Of Combined Criteria - Diagnostic Criteria For Diabetes. Sci J Endo and Meta. 2018; 1(1): 001-006.
79. Maria I Borelli, et al. Effect Of Endogenous Islet Catecholamines Possible Modulatory On Insulin Secretion. Sci J Endo and Meta. 2018; 1(1): 001-005.
80. Louis Irwin, et al. Effect of exercise in combination with dietary nopal and zucchini on chronic and acute glucohomeostasis in genetically obese mice. Inte J Expe Bio. 2018; 1(1): 001-005.
81. Vijaya Saradhi Settaluri, et al. Validation of Non Essential Amino Acids and Total Protein Content in Different Categories of Tea. Inte J Expe Bio. 2018; 1(1): 01-04.
82. Patrick D Craig, et al. T Antigen: Polyomavirus Middle of Natural Biology. Inte J Expe Bio. 2018; 1(1): 001-007.
83. Yoshinori Ohsumi, et al. The HIV Coreceptor CCR5 - Recycling and Endocytosis. Inte J Expe Bio. 2018; 1(1): 001-008.
84. Marino Zerial, et al. Elicitation of the Angiogenic Phenotype1: Transforming Myc Protein for In Vivo. Inte J Expe Bio. 2018; 1(1): 001-008.
85. Zhang Y, et al. Odorant Receptor In Mammali : The Caenorhabditis Elegans Seven-Transmembrane Protein ODR-10 Functions on Cells. Inte J Expe Bio. 2019; 1(1): 001-008.
86. Kazuo Maeda, et al. Improved Outcome with Novel Studies in Fetal Monitoring. Sci J of Gyne and Obste. 2019; 2(1): 001-004.
87. Sunil J. Wimalawansa, et al. Vitamin D Deficiency-Related Reproductive Consequences. Sci J of Gyne and Obste. 2019; 2(1): 001-006.
88. Munch A, et al.Investigation in blood Leukocytes and Neutrophils in Periparturient Dairy Cow. Sci J of Gas and Hepa. 2018; 1(1): 001-006.
89. Jie Song Hua, et al. Primary Helicobacter Pylori Resist

Cite this article: Enzyme Functionality: Zn2+-Chelating Peptide and Reaction of Zinc Enzymes. Sci J Mat Sci & Eng. 2019; 1(1): 001-008.

ance to Clarithromycin and Metronidazole in Singapore. Sci J of Gas and Hepa. 2018; 1(1): 001-003.
90. Paul Moayyedi, et al. A Systematic Review and Economic Analysis: Proton Pump Inhibitors in Nonulcer Dyspepsia Efficacy. Sci J of Gas and Hepa. 2018; 1(1): 001-003.
91. Zhen-Ning Wang, et al.Gastric Cancer: Collagen IV Expression and Biological Behavior. Sci J of Gas and Hepa. 2018; 1(1): 001-002.
92. Zhen-Ning Wang, et al. A Possible Pathophysiologic Contribution to Necrotizing Enterocolitis: Human Intestine Inflammation. Sci J of Gas and Hepa. 2018; 1(1): 001-006.
93. Paul M Wassarman, et al. Egg Interaction during Mammalian Fertilization in the Molecular Basis of Sperm . Sci J of Gyne and Obste 2018; 1(1): 001-006.
94. Mary Lou Moore, et al. Breastfeeding Benefits Support -Research. Sci J of Gyne and Obste 2018; 1(1): 001-002.
95. Pepita Gimenez-Bonafe, et al. Preservation of Fertility in Patients with Cancer. Sci J of Gyne and Obste 2018; 1(2): 001-006.
96. Yueyang F Fei, et al. Non-Hemorrhagic Unilateral Adrenal Infarct In Pregnancy: A Case Report. Sci J of Gyne and Obste. 2019; 1(1): 001-002.
97. Karen Pierre, et al. Protein-Energy Adequacy of Dialysis Patients in Trinidad and Tobago. Am J of Nep and Ther. 2018; 1(1): 01-05.
98. Balakrishna N, Tenckhoff Catheter Surgical under Local Anesthesia. Am J of Nep and Ther. 2018; 1(1): 001-003.
99. J T Ohlsson, et al. Man in angiotensin and noradrenaline inhibits the Endothelin. Am J of Nep and Ther. 2018; 1(1): 001-005.
100. David J, et al. Apoptosis and Ischemic Renal Injury Reduce the Guanosine Supplementation. Am J of Nep and Ther. 2018; 1(1): 001-005.
101. R W Baldeweg, et al. Tumor-induced osteomalacia : Cloning and characterization of Fibroblast Growth Factor 23. Am J of Nep and Ther. 2018; 1(1): 001-006.
102. Amitabh Arya, et al. Post Pyeloplasty Follow Up In Children And Adolescents: Diuretic Renography Or Renal Ultrasonography? Am J of Nep and Ther. 2019; 2(1): 001-005.
103. Amitabh Arya, et al. Post Pyeloplasty Follow Up In Children And Adolescents: Diuretic Renography Or Renal Ultrasonography? Am J of Nep and Ther. 2019; 2(1): 001-005.
104. Richard Lechtenberg, et al. Tau Interferon in Multiple Sclerosis. Amer J Neur & Neurophysi. 2018; 1(1): 001-002.
105. Eva Guy Rodriguez, et al. Discussion of the differential diagnosis of bilateral thalamic lesions-Bilateral thalamic infarcts due to occlusion of the Artery of Percheron. Amer J Neur & Neurophysi. 2018; 1(1): 001-004.
106. Yhashi Chang, et al. IVIg for Miller Fisher syndrome: Cerebral infarction. Amer J Neur & Neurophysi. 2018; 1(1): 001-002.
107. Fredrick J. Seil, et al. T cell responses to Myelin Antigens and Antimyelin Antibodies. Amer J Neur & Neurophysi. 2018; 1(1): 001-005.
108. Y Niimi, et al. Embolization of Spinal Cord AVMs: Neurophysiologic Provocative Testing. Amer J Neur & Neurophysi. 2018; 1(1): 001-002.
109. Ameni Touati, et al. Some Reducibility Results for Differentiable Sets. Amer J Neur & Neurophysi. 2019; 1(1): 001-005.
110. Chrisostomos Sofoudis, et al. Sofoudis C. Septic Abortion Accompanied with Dessiminated Intravascular Coagulation and Acute Cardiomyopathy Presentation of a Rare Case and Mini Review. Am J Nur & Pract. 2018;1(1): 001-00.
111. Nick Jones, et al. Nurse Practitioners and Family Physicians Ethics Health Care Services. Am J Nur & Pract. 2018; 1(1): 001-005.
112. Thomas R A, et al. Human Infants Learning by Prenatal and Postnatal Flavor . Am J Nur & Pract. 2018; 1(1): 001- 006.
113. D. J Wise, et al. A Randomized, Double-Blind, Placebo-Controlled - Milk Production in Mothers of PremaTure Newborns Domperidone Drug effect. Am J Nur & Pract. 2018; 1(1): 001-005.
114. Bronagh Bufton, et al. Effects of Nursing Homes Ownership Compromise the Quality of Care. Am J Nur & Pract. 2018; 1(1): 001-005.
115. Kerstin Ekberg, et al. How Physicians Deal With the Task of Sickness Certification in Cause-Based and Comprehensive Disability Systems – A Scoping Review. Am J Nur & Pract. 2019; 2(1): 01-10.
116. Michael J. Vives, et al. Factors in Choosing the Surgical Approach: Cervical Spondylotic Myelopathy. Am J Orth and Rhe. 2018; 1(1): 001-004.
117. M. Runge, et al. Geriatric Patients in Balance Training and Exercise. Am J Orth and Rhe. 2018; 1(1): 001-003.
118. Ukoha Ukoha Ukoha, et al. Nutrient Foramina in Long Bones : Study. Am J Orth and Rhe. 2018; 1(1): 001-003.
119. Zhiquan An, et al. Human Humeral Diaphysis of the Nutrient Foramina : Anatomical Study : Study. Am J Orth and Rhe. 2018; 1(1): 001-007.
120. K-P Günther, et al. Hip Replacement in Rates: International Variation: Study. Am J Orth and Rhe. 2018; 1(1): 001-005.
121. Saeed Taj din, et al. Level of Physical Activity among Diabetic Patients of Rural and Urban Areas. Am J Orth and Rhe. 2019; 2(1): 001-004.
122. Carolina Caleza Jiménez, et al. Breastfeeding, Bed-Sharing and Early Childhood Caries. Is There An Association? A Review of the Literature. Am J Pedi and Heal care. 2018; 1(1): 001-004.
123. Katarzyna Niewiadomska-Jarosik, et al. Lipid Profile in Children Born As Small for Gestational Age . Am J Pedi and Heal care 2018; 2(1): 01-03.
124. Mustafa Aydin, et al. Antibiotic Susceptibility Pattern and Clinical Features of Klebsiella Sepsis in Newborn Infants. Am J Pedi and Heal care 2019; 1(1): 01-04.
125. H Dele Davies, et al. Necrotizing Fasciitis- Flesh-Eating Bacteria Disease. Am J Pedi and Heal care 2019; 1(1): 01-06.
126. Marie Westwood, et al. The diagnosis of urinary tract infection (UTI) in children under five years: Rapid tests and urine sampling techniques. Am J Pedi and Heal care 2019; 1(1): 01-09.
127. Folkert Fehr, et al. What Entrustable Professional Activities Add To a Primary Care Residency Curriculum. Am J Pedi and Heal care 2019; 2(1): 01-06.
128. Sonya Martin, et al. Spatially Modulated Illumination Microscopy□D measures the size of Biological Nanostructures . Ann of Phar Nano Tech and Nanomedi. 2018; 1(1): 01-05.
129. Sonya Martin, et al. Genetic analysis of Fis interactions with their binding sites. Ann of Phar Nano Tech and Nanomedi. 2018; 1(1): 01-07.
130. John H Reif, et al. Nucleation assembly of DNA tile complexes is directed by barcode-patterned lattices. Ann of Phar Nano Tech and Nanomedi. 2018; 1(1): 01-07.
131. Thomas H LaBean, et al. Self assembly of DNA nanotubes from triple-crossover tiles as templates for conductive nanowires. Ann of Phar Nano Tech and Nanomedi. 2018; 1(1): 01-05.
132. Ulrich Kettling, et al. Dual-Photon Fluorescence Coincidence Analysis: Rapid quantification of Enzyme activity. Ann of Phar Nano Tech and Nanomedi. 2018; 1(1): 01-05.
133. Ahmed R. Gardouh, et al. Design, Optimization and In-Vitro Evaluation of Antifungal Activity of Nanostructured Lipid Carriers of Tolnaftate Ann of Phar Nano Tech and Nanomedi. 2019; 2(1): 01-05.
134. Mohammed Khalid, et al. Khalid M. Predictors of Prognosis in Pulmonary Hypertension. Anna Pul and Crit Car Med.

Cite this article: Enzyme Functionality: Zn2+-Chelating Peptide and Reaction of Zinc Enzymes. Sci J Mat Sci & Eng. 2019; 1(1): 001-008.

2018; 1(1): 001-004.
135. Abdullah Alsaeedi, et al. The Prevalence of Smoking among sample of Kuwait Asthmatics and its impact on the response of the treatment, Anna Pul and Crit Car Med. 2018; 1(2): 001-002.
136. Nicolau Beckmann, et al. Resolving the Oedematous Signals Induced by OVA Challenge in the Lungs of Actively Sensitised Rats. Anna Pul and Crit Car Med.. 2018; 1(1): 01-06.
137. Thomas J walsh, et al. Investigate the performance of non-invasive diagnostic tests such as galactomannan enzyme immunoassay and quantitative Caspofungin in the early diagnosis of invasive aspergillosis (IA). Anna Pul and Crit Car Med.. 2018; 1(1): 01-06.
138. Charles B. Huddleston, et al. Lung Transplantation in pediatrics. Anna Pul and Crit Car Med.. 2018; 1(1): 01-05.
139. Jeffrey P. Lamont, et al. Comparision of valved vs non-valved implantable ports for vascular access:A randomized trial. Anna Pul and Crit Car Med.. 2018; 1(1): 01-03.
140. D Inwald, et al. Risk and relevance of open lung biopsy in Nonneonatal extracorporeal membrane oxygenation (ECMO) patients. Anna Pul and Crit Car Med.. 2018; 1(1): 01-04.
141. Guillaume Mortamet, et al. Does Esophageal Pressure Monitoring Reliably Permit To Estimate Trans pulmonary Pressure In Children?. Anna Pul and Crit Car Med.. 2018; 2(2): 01-05.
142. Yang Jin, et al. Extracellular Vesicle-Shuttling MicroRNAs Regulate the Development of Inflammatory Lung Responses. Anna Pul and Crit Car Med.. 2018; 1(2): 01-04.
143. Nicola Clemente, et al. Pneumonectomy As A Salvage Therapy: A Rare Indication For A Gastric Malt Lymphoma Disseminated To The Lung. Anna Pul and Crit Car Med.. 2018; 1(2): 01-04.
144. Nicola Clemente, et al. Pneumonectomy As A Salvage Therapy: A Rare Indication For A Gastric Malt Lymphoma Disseminated To The Lung. Anna Pul and Crit Car Med.. 2018; 1(2): 01-04.
145. Victor Chew, et al. Pulmonary Cement Embolism. Anna Pul and Crit Car Med. 2019; 2(1): 01-02.
146. Victor Chew, et al. An Unusual Cause of a Tension Pneumothorax. Anna Pul and Crit Car Med. 2019; 2(1): 01-03.
147. Mark C. Lavigne, et al. A Performance Summary of Agents Used in Oral Care for Non-Ventilated and Mechanically-Ventilated Patients. Anna Pul and Crit Car Med. 2019; 2(2): 01-34.
148. Elisangela Hermes, et al. Psychomotricity in Vestibular Dysfunction Therapy (VDT): A Collective Health Question. Am J Rhin and Otolo. 2018; 1(1): 001- 005.
149. Ramtej J Verma, et al. Diethanolamine-Induced Hepatic Injury and Its Amelioration by Curcumin. Am J Toxi and Res. 2018; 1(1): 001-004.
150. Chee Kong Yap, et al. A Preliminary Screening of Cd and Pb Concentrations in the Some Traditional Chinese Herbal Medicines Bought From Selected Shops in Peninsular Malaysia. Am J Toxi & Res. 2018; 1(1): 001-004.
151. Geza Bozoky, et al. Acute Silent Non-Massive (submassive) Pulmonary Embolism. Am J Ang and Surg . 2018; 1(1): 001-003.
152. Muhammad Imran Qadir, et al. Is Hunting Lovering Associates with Pulse Rate. Am J of Viro and Dis. 2019; 1(1): 01.
153. Mujahid Rasheed, et al. Relation of Blood Group with Motion Sickness. Am J of Viro and Dis. 2019; 1(1): 02.
154. Mujahid Rasheed, et al. Views of University Paramedical Students about Causes of Pharyngitis, Its Transmission and Medicinal Control. Am J of Viro and Dis. 2019; 1(1): 02.
155. Kainat Rafaqat, et al. Views of University Paramedical Students about Causes of Pharyngitis, Its Transmission and Medicinal Control. Am J of Viro and Dis. 2019; 1(1): 02.
156. Sajid Ullah, et al. HCV Prevalence in the Volunteer Blood Donors in District Bajaur Khyber Pakhtunkhwa Pakistan. Am J of Viro and Dis. 2019; 1(1): 02.
157. Rabbia Aslam, Analogue of Breathing With Lizard Fright Am J of Viro and Dis. 2019; 1(1): 01.
158. Hurain Shaukat, et al. Linkage of Body Temperature with Exercise Am J of Viro and Dis. 2019; 1(1): 01.
159. Mariyam Javed, et al. How Breathe Rate Relates With Cricket Likeness? Am J of Viro and Dis. 2019; 1(1): 02.
160. Hakan Alfredson, et al. Achilles and patellar tendon operations performed in local anestesia, Am J Anest and Pai med. 2018; 1(1): 001-002.
161. Richard Lechtenberg, et al. Tau Interferon in Multiple Sclerosis. Amer J Neur & Neurophysi. 2018; 1(1): 001-002.

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