World Journal of

Methodology

World Journal of *Methodology*

A peer-reviewed, online, open-access journal of methodology

Editorial Board

2011-2015

The *World Journal of Methodology* Editorial Board consists of 238 members, representing a team of worldwide experts in methodology. They are from 41 countries, including Argentina (2), Australia (7), Austria (3), Belgium (4), Brazil (3), Canada (10), China (27), Croatia (1), Cuba (1), Czech (3), Denmark (2), Egypt (1), France (6), Germany (4), Greece (3), Hungary (2), India (8), Iran (3), Israel (1), Italy (18), Japan (12), Lithuania (1), Malaysia (1), Mexico (2), Netherlands (2), New Zealand (1), Norway (3), Pakistan (2), Poland (2), Portugal (3), Romania (4), Russia (2), South Korea (3), Spain (17), Sweden (1), Thailand (2), Turkey (2), United Arab Emirates (1), United Kingdom (10), United States (57), and Uruguay (1).

Hans Sanderson, *Roskilde*

Egypt

Nervana S Bayoumi, *Cairo*

France

Marc Y Bardou, *Dijon*
Mohammed M Bettahar, *Nancy*
Olivier David, *Grenoble*
Florian Lesage, *Sophia Antipolis*
Patrick Maison, *Creteil*
Sandrine Marquet, *Marseille*

Germany

Harald Hampel, *Frankfurt/ Main*
Frank Peinemann, *Cologne*
M Lienhard Schmitz, *Giessen*
Alfons Schnitzler, *Duesseldorf*

Greece

Konstantinos P Economopoulos, *Athens*
Demosthenes Panagiotakos, *Athens*
Issidora Papassideri, *Athens*

Hungary

Péter Halász, *Budapest*
András Komócsi, *Pécs*

India

Dipshikha Chakravortty, *Bangalore*
DK Dhawan, *Chandigarh*
R Jayakumar, *Cochin*
Abdul Viqar Khan, *Aligarh*
Geetha Manivasagam, *Vellore*
Jacob Peedicayil, *Vellore*
YS Prabhakar, *Lucknow*
Rakesh Kumar Sinha, *Ranchi*

Iran

Mehran Javanbakht, *Tehran*
Enayat Kalantar, *Sanandaj*
Shekoufeh Nikfar, *Tehran*

Israel

Dan Frenkel, *Tel Aviv*

Italy

Giuseppe Biondi-Zoccai, *Modena*
Carlo Bonanno, *Vicenza*
Paolo Borrione, *Turin*
Filippo Cademartiri, *Monastier di Treviso*

Alberto Chiesa, *Bologna*
Annamaria Cimini, *L'Aquila*
Giovanni Di Leo, *San Donato Milanese*
Giovanna Ferraioli, *Milan*
Irene Floriani, *Milan*
Landoni Giovanni, *Milano*
Stefano Girotti, *Bologna*
Paola Irato, *Padova*
Mario Mascalchi, *Florence*
Patrizia Mecocci, *Perugia*
Germano Orrù, *Cagliari*
Carlo Riccardi, *Perugia*
Mauro Valtieri, *Rome*

Japan

Kohei Akazawa, *Niigata*
Subash CB Gopinath, *Tsukuba*
Koichi Hattori, *Tokyo*
Satoshi Hirohata, *Okayama*
Masahiro Kohzuki, *Sendai*
Yoshinori Marunaka, *Kyoto*
Kenji Miura, *Tokorozawa*
Ryuichi Morishita, *Suita*
Mitsuhiko Noda, *Tokyo*
Yurai Okaji, *Tokyo*
Hirosato Seki, *Osaka*
Hisanori Umehara, *Kahoku-gun*

Lithuania

Giedrius Barauskas, *Kaunas*

Malaysia

Iis Sopyan, *Kuala Lumpur*

Mexico

Martha Rodríguez-Moran, *Durango*
Julio Sotelo, *Mexico*

Netherlands

Bart J Polder, *Emmeloord*
Frank Twisk, *Limmen*

New Zealand

Valery Feigin, *Auckland*

Norway

Cato Grønnerød, *Fredrikstad*
David F Mota, *Oslo*
Tore Syversen, *Trondheim*

Pakistan

Muhammad A Noor, *Islamabad*
Yasir Waheed, *Islamabad*

Poland

Piotr Dziegiel, *Wroclaw*
Tadeusz Robak, *Lodz*

Portugal

Nuno Lunet, *Porto*
Hugo Sousa, *Porto*

Romania

Elena Moldoveanu, *Bucharest*
Monica Neagu, *Bucharest*
Florin-Dan Popescu, *Bucharest*

Russia

Galina B Bolshakova, *Moscow*
Sergey V Dorozhkin, *Moscow*

South Korea

Sang Soo Hah, *Seoul*
Chang-Yong Lee, *Kongju*

Spain

Salvador F Aliňo, *Valencia*
Mohamed Farouk Allam, *Cordoba*
Alejandro Cifuentes, *Madrid*
Miren Lopez de Alda, *Barcelona*
Joaquin de Haro, *Madrid*
Emma Garcia-Meca, *Cartagena*
Mónica H Giménez, *Zaragoza*
M de la Guardia, *Valencia*
Josep M Guerrero, *Barcelona*
Fernando Marin, *Madrid*
José A Orosa, *A Coruña*
Jesús Osada, *Zaragoza*
Soledad Rubio, *Córdoba*
Helmut Schröder, *Barcelona*
Jesus Simal-Gandara, *Ourense*
Gabriela Topa, *Madrid*
Miguel A Vallejo, *Madrid*

Sweden

Jenny Selander, *Stockholm*

Thailand

Amporn Jariyapongskul, *Bangkok*
Bungorn Sripanidkulchai, *Khon Kaen*

Turkey

Ferda E Percin, *Ankara*
Aysegul Yildiz, *Izmir*

World Journal of *Methodology*

Contents

Contents

ABOUT COVER

World Journal of Methodology Editorial Board, Laurentiu M Popescu, Professor, Department of Cellular and Molecular Medicine, School of Medicine, Carol Davila University of Medicine and Pharmacy, 050474 Bucharest, Romania

AIM AND SCOPE

World Journal of Methodology (*World J Methodol*, *WJM*, online ISSN 2222-0682, DOI: 10.5662) is a bimonthly peer-reviewed, online, open-access (OA), journal supported by an editorial board consisting of 23 experts in methodology from 41 countries.

WJM aims to rapidly report the most recent results in medical diagnostics, therapeutic techniques and equipment, clinical medical research, clinical and experimental techniques and methodology. It provides a platform to facilitate the integration of clinical medicine and experimental techniques and methodology to help clinicians improve diagnostic accuracy and therapeutic efficacy. The journal publishes original articles and reviews on the following topics: (1) Clinical medical techniques, including but not limited to those for pharmaceutical medicine, laboratory medicine, radioactive medicine, medical imaging, nuclear medicine, physical therapy, pathology, surgery, disinfection, nutritional therapy, transfusion and medical equipment; (2) Clinical medical research on etiology, epidemiology, pathogenesis, morphology and function, signs and symptoms, clinical trials, and evidence-based medicine; and (3) Laboratory methodology, including but not limited to techniques in DNA/RNA sequencing, preparation and transformation of competent cells, PCR, protein biochemistry, cell biology, genetics and epigenetics, immunology, microbiology, animal models of human pathologies, bioinformatics, and laboratory equipment manipulation and control.

Protein dynamics *via* computational microscope

Anton B Guliaev, Senping Cheng, Bo Hang

Anton B Guliaev, Department of Chemistry and Biochemistry, San Francisco State University, San Francisco, CA 94132, United States
Senping Cheng, Pan-Pacific Biopharma, Inc., San Francisco, CA 94105, United States
Bo Hang, Department of Cancer and DNA Damage Responses, Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, United States
Author contributions: Guliaev AB is a primary and the corresponding author for the manuscript; Cheng S and Hang B are co-authors and contributed to the discussion and provided critical reading of the manuscript.
Correspondence to: Anton B Guliaev, Assistant Professor, Department of Chemistry and Biochemistry, San Francisco State University, San Francisco, CA 94132,
United States. guliaev@sfsu.edu
Telephone: +1-415-4052961 **Fax:** +1-415- 3382384
Received: September 29, 2012 **Revised:** November 19, 2012
Accepted: November 28, 2012
Published online: December 26, 2012

Peer reviewer: Line Pedersen, PhD, Centre of Inflammation and Metabolism, Rigshospitalet, Faculty of Health Science, Copenhagen University, Blegdamsvej 9, 2100 København, Denmark

INTRODUCTION

The understanding of functions of biological macromolecules has relied not only on the structural data but also their dynamical behavior. The early view of the proteins as relatively rigid structures has been replaced by a dynamic model. In these models conformational changes and motions play an essential role in their function. In the last 25 years, biomolecular simulations has become a powerful tool to trace atomic motions of protein and DNA molecules and yielded important insights into molecular mechanisms[1-3]. The molecular dynamics (MD) simulation is considered a pivotal methodology to access dynamical behavior of macromolecules *in silico*. This technique provides ultimate atomic resolution details about the dynamic properties of the system as a function of time. It offers connection between the structure and function by enabling the exploration of the conformational space and energy landscape available to protein molecules. It is not surprising that the term "computational microscope" has been accepted by the scientific community[4].

One of the first MD simulations of proteins, bovine pancreatic trypsin inhibitor (BPTI), was reported in 1977. This work should be considered as the first attempt to utilize computer simulation to observe protein motions at 9.2 picosecond (ps) time scale[5]. In 1988, eleven years later, the same protein was simulated for 210 ps using explicit solvent[6]. The latter work showed good correlation between the average structure produced by simulation and the high-resolution X-ray structure, emphasizing the importance of longer simulation

Abstract

The purpose of this overview is to provide a concise introduction to the methodology and current advances in molecular dynamics (MD) simulations. MD simulations emerged as a powerful and popular tool to study dynamic behavior of proteins and macromolecule complexes at the atomic resolution. This approach can extend static structural data, such as X-ray crystallography, into dynamic domains with realistic timescales (up to millisecond) and high precision, therefore becoming a veritable computational microscope. This perspective covers current advances and methodology in the simulation of protein folding and drug design as illustrated by several important published examples. Overall, recent progress in the simulation field points to the direction that MD will have significant impact on molecular biology and pharmaceutical science.

time and use of explicit water molecules. Since then the dramatic increase in computational power and significant improvement in methodology made the simulation much more accurate and stable. It is now routine to run simulations from 100 ns (10^{-7} s) to several μs (10^{-6} s) where even shorter time scales (10-100 ns) can well describe local dynamics of the proteins structures. Experimental measurements show that rotation of protein side chains often occurs around 0.5 ns timescale ["Physical Biology of the Cell", Rob Phillips, Jane Kondev and Julie Theriot (2009) and references therein]. However, since many functionally important biological events occur on timescales of many microseconds or milliseconds, including protein folding, protein-protein or protein-drug interactions, much longer time scales are needed for conformational sampling. Recently, there were a large number of efforts to extend simulations to a longer timescales by designing specialized hardware to speed up MD simulations and/or by improving the code[7-9]. The longest MD simulation published to date is a millisecond (ms) [1000 microsecond (μs)] simulation of the BPTI protein on a special-purpose supercomputer (called Anton) designed for MD simulations[10-12]. This work showed that BPTI transitioned reversibly among a small number of structurally distinct states with two states in the simulation accounting for 82% of the trajectory which agreed well with experimental data. Such behavior could not be observed at shorter (μs) timescales and thus confirms the importance of adequate simulations times.

The MD relies on the solution of the equations that describes the motion of the molecular system. In explicit, all-atom simulations, thousands to millions of individual atoms, representing, for instance, all atoms of the protein and surrounding water molecules, move in a series of short [e.g., 2 femtoseconds (fs)], discrete time steps. The MD trajectory represents the sequential set of time-evolved snapshots of atomic coordinates and generally provides data only at the level of atomic positions, velocities and single-point energies. The application of statistical thermodynamics allows to obtain macroscopic properties such as pressure, heat capacity and free energies of the system. The product is a detailed "movie" of molecular behavior over time. As a result, MD has became a tool for researches to investigate structural aspects, kinetics and thermodynamics of various macromolecule systems, including the dynamics in enzyme activity[13-15], transport of ion and small molecules[16-18], protein-protein and protein-DNA interactions [19-21], protein folding[22,23] and ligand-target interactions[24-27], just to mention a few. In general, each step in MD simulations consists of computationally intensive force calculations, followed by a less expensive integration step that advances the positions of the atoms according to classical laws of motions. Forces for each atom of the system are calculated based on the molecular mechanics equations. The functional form of such equations is shown in Figure 1. The potential energy parameters (bond lengths, vibrational energies, force constants, atomic and partial atomic charges, proper van der Waals atomic radii, *etc.*)

are derived empirically from experimental techniques such as spectroscopy and X-ray crystallography, and also from ab initio quantum mechanical calculations. Collectively, these parameters describe the contributions of various atomic forces that govern MD and are called a 'force field'. There are several well established force fields which are currently being used and continued to be developed. The Chemistry at HARvard Macromolecular Mechanics (CHARMM) biomolecular force field developed by Karplus *et al*[28,29] is widely used for the simulation of proteins and other many particle systems using CHARMM and NAMD molecular simulation programs[30]. The Assisted Model Building with Energy Refinement (AMBER), developed by the Kollman group[31], is a set of molecular mechanical force fields for the simulation of biomolecules, which was initially developed for the nucleic acids and now adopted for the simulation of a wide range of the biomolecules, including proteins. AMBER has a package of molecular simulation programs and can also be used by NAMD. There are several others that are available, including the Energy Calculation and Dynamics (ENCAD) developed by Levitt [32], the GROMOS and the hydrocarbon force fields (MM2-4)[33]. ENCAD and CROMOS force fields are available through the Groningen Machine for Chemical Simulations computational package[7]. In general, the force fields express the total force on an atom as a sum of 3 major components: bonded forces which involve interactions between small groups of atoms connected by one or two covalent bonds; van der Waals forces which include interactions between small groups of atoms in the system; and electrostatic forces which include interactions between all pairs of atoms (non-bonded pair-wise interactions) (see the functional form of AMBER equation, Figure 1). Chemical bonds and atomic charges are modeled using simple harmonic motions, and dihedral angles are modeled using a sinusoidal function that approximates the energy differences between eclipsed and staggered conformations. The non-bonded forces arise from van der Waals forces and electrostatic interactions. The non-bonded energy terms between every pair of atoms increase as the square of the number of atoms for a pairwise model (N^2) and represent largest summation term in the potential energy equation. This part of the simulations is the most computationally expensive. The van der Waals forces fall quickly with the distance and in computer simulations are treated with 12/6 Lennard-Jones potentials. To reduce the cost of calculations the contributions from these forces can be controlled by the user defined cutoff distance. The very short cutoff (-4 Å) does not adequately model the properties of the system when a very long cutoff (over 20 Å) significantly increases simulation time and has been shown not to improve fidelity of the simulation over the intermediate values around 9-12 Å which are commonly used in the modern simulation[34]. The electrostatic forces fall off slowly with distance and truncation at cutoff distance introduces significant errors in the simulations. Electrostatic forces, modeled using Coulomb's

law, are typically computed using approximate methods (such as Ewald summations) that account for long-range effects with the explicit interaction of all pairs of atoms[35,36]. However, recent studies showed that use of approximation methods could affect the results of the simulations. For example, in case of the protein folding using short (9 Å) cutoff distances could shift the balance between hydrophobic and hydrophilic interactions leading to stabilization of the more compact structures[37]. Moreover, the neglect to include long-range component of the Lennard-Jones interactions and the lack of highly accurate treatment of the long-range electrostatics could result in artifacts during the folding pathways. In contrary, the free energy of the protein folding and structural properties of the folded protein are relatively unaffected by these approximations[37]. The level of the approximations used in MD simulations should depend in part on the goal and questions that these calculations are planned to address.

The common protocol for performing MD simulations consists of a number of steps and is shown in Figure 2. In simulations of biomolecules, an X-ray crystal structure or an nuclear magnetic resonance (NMR) structure is first obtained from the Brookhaven Protein Databank to be commonly used as the initial structure. It is also possible to use a theoretical structure developed by homology modeling (also known as comparative modeling) or extended protein sequence if the researcher desires to investigate protein folding. Prior to starting MD simulation, it is recommended to perform energy minimization of the structure to remove any strong van der Waals interactions which might exist in the initial structure. These unwanted interactions can lead to local structural distortion resulting in an unstable simulation. At this point, explicit water molecules are added to solvate the system. Another energy minimization with the macromolecule fixed should follow the solvation step to allow the added water molecules to readjust around the protein molecule. At this point, the initial velocities at a low temperature are assigned to each atom of the system and Newton's equations of motion are integrated to propagate the system in time. In explicit solvent simulation, the protein position is fixed initially to allow equilibration of water molecules. Once that is done, the constraints on the protein can be removed and the whole system (macromolecule system and waters) can evolve in time. At the beginning of simulations velocities are assigned at low temperature and simulation proceeds *via* heating phase. Periodically, if needed, new velocities are assigned at a slightly higher temperature and the simulation is allowed to continue. This is repeated until the desired temperature is reached (commonly around 300-310 K). Once the desired temperature is reached, the equilibration simulation of the system continues and properties such as structure, pressure, temperature and energy are monitored. The purpose of equilibration dynamics is to run the simulation until these properties become stable with respect to time. If the temperature increases or decreases significantly, the velocities can be

scaled such that the temperature returns to near its desired value. The final step of the simulation is to run the simulation in "production" phase for the desired time length. This can be from several to 10^6 ns. The MD trajectories from the production dynamics can be analyzed and post-processed to obtain conformational dynamics, thermodynamic parameters and free energy values for the system of interest. There are many modifications of the procedure described above using different protocols to control the temperature and generate MD trajectories. The additional details on MD procedures and theory can be found elsewhere[38-40]. The new methods such as replica-exchange MD (REMD) have been developed and employed for the simulation of macromolecules[41]. A series of replicas are run in parallel at temperatures ranging from a desired temperature to a high temperature at which the replica can easily overcome small energy barriers[42]. The REMD approach can overcome the multiple-minima problem known to trap structures in the local conformational minimum by exchanging non-interacting replicas of the system at several temperatures.

The vast increase in the number of MD papers published in the last 10 years, including publications in those highly respected journals such as Nature and Science, is a clear evidence that MD simulations become a widely accepted tool for investigation of conformational features for biological macromolecules. The number of publications using MD is now in tens of thousands. For example, using "molecular dynamics" and "proteins" as topic words to search ISI "web of science" (http://apps.webofknowledge.com/) yielded almost 4000 papers in 2011 *vs* about 1200 articles in 2001. Therefore, it is impossible for such a short review to adequately describe all major work done using MD simulations. Here we chose to describe a small number of specific examples to illustrate the use of the MD simulations to obtain functionally relevant information.

MD AND PROTEIN FOLDING

Understanding the protein folding or how the polypeptide chain is able to fold to its native conformation is essential to the description of the function of the protein. It is well established that misfolding is present in a variety of diseases[43]. One of the key aspects of the protein folding is the mechanisms by which the protein finds the native conformation despite enormous number of statistically possible conformations. In addition, the folding is slow on the simulation time scale with many proteins taking microseconds to fold. So far only small sized proteins (10 to 80 amino acid residues), with no disulfide bonds or prostatic groups, have been successfully folded to its native conformation. The small sizes and rather short folding times of such proteins make them good candidates for MD simulation using all-atom models in explicit solvent since the native conformation of these proteins is determined solely by the protein amino acid sequence[44]. The recent work from the DE Shaw group[45] summarized the simulations of 12 proteins

$$V = \sum_{bonds} k_b(l-l_o)^2 + \sum_{angles} k_a(\theta-\theta_o)^2 + \sum_{torsions} \frac{1}{2}V_n[1+\cos(n\omega-\gamma)] +$$

$$\sum_{j=1}^{N-1}\sum_{i=j+1}^{N}\left\{ \varepsilon_{i,j}\left[\left(\frac{r_{o,ij}}{r_{ij}}\right)^{12} - 2\left(\frac{r_{o,ij}}{r_{ij}}\right)^{6}\right] + \frac{q_i q_j}{4\pi\varepsilon_o r_{i,j}}\right\}$$

Figure 1 An example of the equation used to calculate atomic forces.
The equation describes potential energy in the Assisted Model Building with Energy Refinement force field. The first summation term represents the energy between the covalently bonded atoms. Summing of the angles, second term, represents the energy due to the angle geometry. Third term represents the energy of the torsion angles. Forth term accounts for the energy of the non-bonded interactions between all pairs of atom. This term can be decomposed into van der Waals and electrostatic energies. The subscript zero represents equilibrium values. The energy terms are parameterized to fit experimental (for example, spectroscopic) data and/or quantum-mechanical calculations.

which represent three major structural classes (α-helical, β-sheet and mixed α/β). All simulations started from extended conformations to avoid bias toward the native state. In that work total of approximately 8 ms of MD simulations were collected. Figure 3 shows the representative structures of those 12 proteins. For each protein MD folded structure is shown in blue and superimposed on the experimental determined structure. For most of the structures (8 out of 12) the root mean square deviations of Cα atoms (Cα-RMSDs) between the two structures, which includes flexible tails residues, were below 2 Å, indicating an excellent agreement between experiment and simulation. To confirm the finding of this work, simulations were performed near the melting temperature, at which both unfolding and folding could be observed repeatedly bringing total to 10 unfolding and 10 folding events for each protein[45]. The authors collected about 400 folding and unfolding events for a total of 8 ms of simulation. The proteins exhibiting larger RMSD values (BBL, protein B, homeodemain and α3D) all contain 3 α-helices. It is possible to suggest that this could indicate minor imperfections of the force-field, however, it is also possible to assume that the experimentally determined conformations for these proteins depend on experimental conditions[46,47]. Deviations between the MD and experimental data might represent differences between the protein's structure at the simulated temperature and the lower temperatures used for experimental structure determination. Based on the MD simulations reported by Lindorff-Larsen *et al*[45], the authors were able to propose general principles that underline protein folding. They concluded that the pathway toward the native conformation occurs *via* formation of the key long-range contacts at the early stages of the folding. These contacts support secondary structure elements which are only formed in the unfolded state. Nine out of 11 proteins simulated in that work share more than 60% of the native contacts formed at any given time during the folding process. The formation of number of structural elements was observed in the similar order, suggesting a common folding pathway for the proteins studied. It is reasonable to propose that heterogeneous folding process for various proteins share a large fraction of
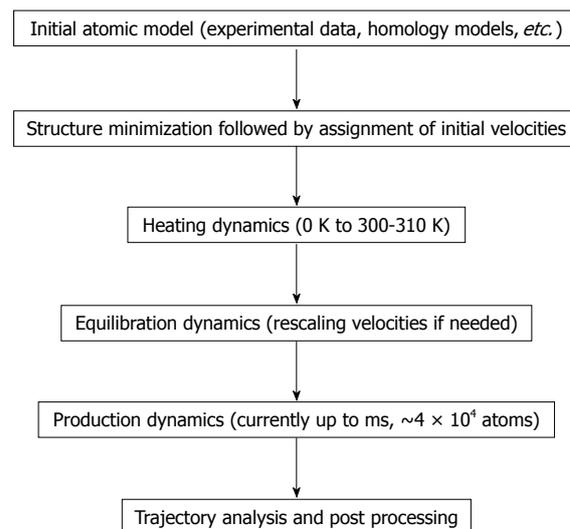
Figure 2 General steps used in molecular dynamics simulations. During the simulations molecular focus on each atom are calculated based on the equation shown in Figure 1. During dynamics calculations (heating, equilibration and production) the positions of atoms are moved according to Newton's law of motion. The simulation time is advanced, and the process is repeated numerous times to generate molecular dynamics rajectory. The longest trajectory is generated during the production run.

structural features and could belong to the same folding "route". The examination of the thermodynamics and kinetics of the folding process showed the absence of a distinct free-energy barrier for folding and the analysis yielded multiple barriers along the folding path which were less than 4.5 k$_B$T. Under experimental conditions corresponding to those used in above simulations folding of the protein was also not determined by single, well-defined free-energy barrier.

Recently, an all-atom MD simulations in explicit solvent, were used to describe the folding path of the 80 amino acid λ-HG mutant of λ-repressor fragment[48]. Using the temperature-dependent MD runs the authors showed that the folding of the λ-repressor is not a two-state process as suggested before. In addition, the authors proposed possible mutation sites which could lead to a fast -folding mutant.

The results described above demonstrated that the current molecule mechanics force fields and simulations protocols are accurate and reliable to make long-time scale MD simulations as a powerful tool for characterizing large conformational changes in proteins.

MD AND DRUG DISCOVERY

For the past 20 years drug discovery has already heavily relying on the computational methods. It is reasonable to predict that computational methods, such as MD simulation, will further advance drug-optimization and enhance our ability to select and design better molecules for the interaction with specific protein targets. It is well established that molecular recognition and drug binding are a very dynamic process. In solution, when a small molecule approaches a target, it deals with the macromol-
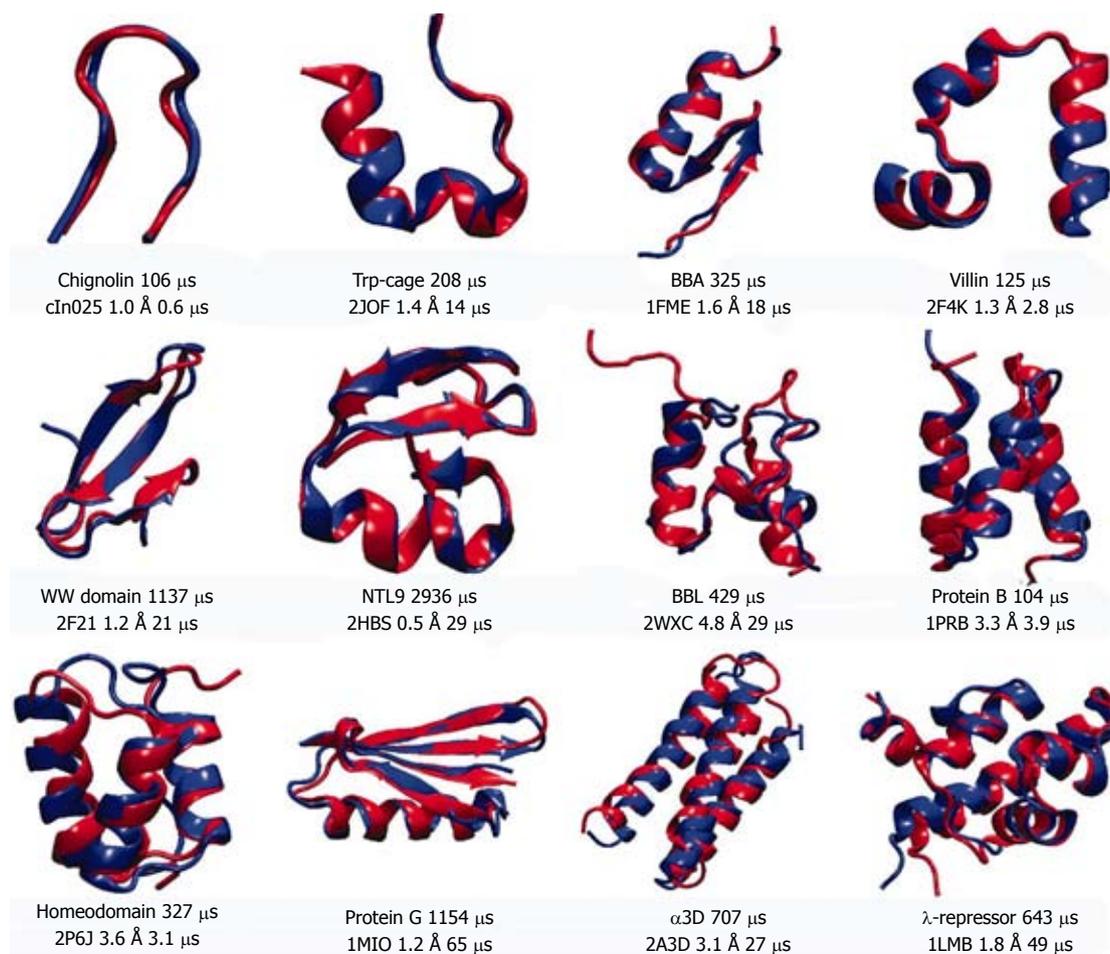
Figure 3 Representative structures of 12 proteins used in molecular dynamics simulation studies[42]. Experimentally determined structures are shown in red and superimposed onto the structures obtained from molecular dynamics simulations (shown in blue). Protein Databank (PDB) entry, root mean square deviations of Cα atoms between two structures and simulation times (total and needed for folding) are displayed for each protein under the superimposed structures. For Protein G the PDB entry was used for the closest homolog, since the structure for the simulated sequence has not been solved experimentally. Reproduced with permission (Copyright 2011, Science publishing group).

ecule in constant motions. Given that the static models produced by X-Ray crystallography and time-averaged structures by NMR tell little about protein dynamics, MD simulation can provide the dynamic picture of the interaction between the small molecule (such as a drug) and the target molecule. Upon binding, the ligand molecule may further induce conformational changes that are not typically sampled when the ligand is absent[49]. There are a number of examples in which binding pathways were successfully reproduced using MD simulations. The complete reconstruction of the binding process of the benzamidine molecule (a known inhibitor of serine proteases) to trypsin was achieved using 495 MD simulations of 100 ns each[50]. Out of 495 trajectories, 187 (37% of total) produced binding events with RMSD less than 2 Å from the crystallographic pose. The obtained binding paths revealed two metastable intermediate states (S2 and S3, Figure 4). The simulation showed that the inhibitor rolls on the surface of the protein prior to entry into the binding pocket (S3 to S4 transition, Figure 4). Using MD simulations the authors estimated the standard free energy of binding which was within 1 kcal/mol from

the experimental value. Reconstruction of the protein-ligand binding process and identification of the transition states suggest that mutations to the transition-state residues may alter the binding kinetics of the inhibitor. Simulations of the binding of several β-blockers to two β-adrenergic receptors (G-protein-coupled receptor modulators, which constitute one-third of all marketed drugs) showed the atomic details of this pharmaceutically critical process in which drug molecules spontaneously bind to the G-protein-coupled receptors to achieve final poses matching those determined crystallographically[51]. The MD revealed dehydration of the ligand and receptor along the binding pathway that represents unexpected kinetic barrier to binding. Such work suggested how receptor/ligand dehydration, known to be a major factor for ligand affinity, might be modulated to affect drug binding kinetics. MD simulation can also be used to determine relative binding free energies between the drug candidate and target protein using Poisson-Boltzmann surface area methods[52,53].

Besides identification of pathways and kinetics of binding, MD simulation can be used to obtain the struc-

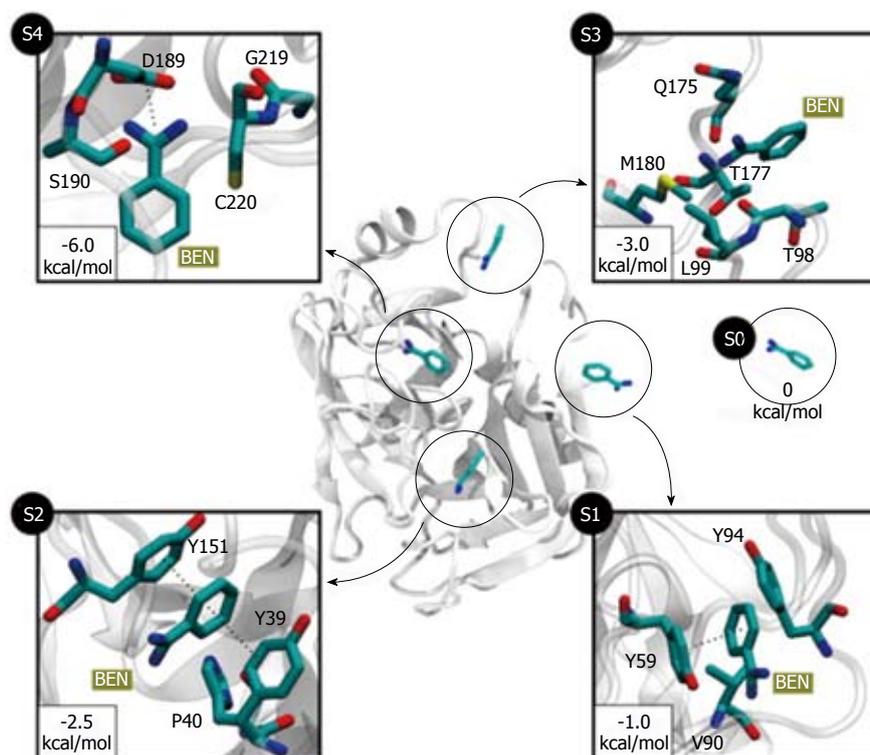**Figure 4 Five different metastable states (S0 to S4) identified for the benzamidine-trypsin complex[47].** The relative free energy between the unbound S0 and the bound S4 states is -6 kcal/mol. The most probable transition to the bound state S4 is from S3, since the barrier between two states is just 1.5 kcal/mol. In states S1 and S2, benzamidine is stabilized by π-π stacking interactions with Y151 and Y39 side chains. In S3, a hydrogen bond may be formed between the NH2 groups of benzamidine (only heavy atoms shown for clarity) and Q175 side chain, or by a cation-π interaction between the Q175 side chain and the benzamidine's benzene ring. Reproduced with permission (Copyright 2011, PNAS).

ture of the target using folding simulations, especially in cases where the structure of the target can not be obtained experimentally. Alternatively, simulation may be used to prepare homology models.

FUTURE DIRECTIONS

Since the millisecond barrier has been broken in MD, it is more than likely that we will be able to make testable predications about the key molecular processes at the atomic levels. Based on the current progress of all-atom MD simulations, a dramatic increase in the length of the generated trajectories should be expected in the nearest future. Parallel to the increase in the timescales accessible to the simulations, there has also been a growth in the size of the systems that can be studied. Much larger systems than those mentioned above have been already tested using MD simulations. These systems are comprised of several million atoms, such as bacterial ribosomes and viruses. In recent studies explicit solvent MD simulations showed essential conformational motions in the ribosome needed for the binding of aminoacyl-tRNA[54]. Such a system consists of 3.2 million atoms. This work showed that conformational motions of the 3'-CCA end of aminoacyl-tRNA resists simple accommodation (single lock-step mechanism), leading to a multistep accommodation process. The proposed mechanism and observed interaction pathways help to interpret large

number of biochemical data and demonstrate that conformational changes during translation occur through a trial-and-error process, rather than in concerted lock-step motions. The 1 million atoms simulation of satellite tobacco mosaic virus (STMV) showed that the empty STMV capsid is not stable, thus explaining the failure of experimental efforts to prepare such empty capsids[55].

Although the conformational changes in the smaller system can be well described using MD simulations, the larger systems (5 millions of atoms or more) still pose many challenges. First, the biologically relevant timescales tend to increase as the system size increases. Second, the high-resolution structures are only available for relatively few very large macromolecule complexes[56]. The computational power to carry out simulation increases can be related to the Moore's law. This law describes how the performance of processors has been increasing exponentially over the last 50 years by doubling approximately every 2 years. Based on the application of the Moore's law to biological systems and without taking into account the design of special hardware architecture for MD, it has been proposed that for every 10-15 years in the past there was an increase of about three orders of magnitude in the timescale and about one and half orders of magnitude in the system size used by MD simulations[57]. Continuation of Moor's law suggests that processor computational power may increase by 1000 fold in the next 10-15 years. In addition

to enhanced processor integration, software and hardware advances will yield further increases. For example, recent application of the Graphic Processing Units (GPUs), together with the development of the general purpose GPU programming model called NVIDIA's Compute Device Architecture, can significantly increase simulation times at fraction of CPU's cost and energy consumption. GPUs have been an integral part of personal computers for decades and their development and low cost has been strongly influenced by the entertainment industry. It has recently been shown that GPUs, due to their unique multiprocessor architecture, can greatly outperform their CPU equivalents. MD simulations can take advantage of GPU acceleration and achieve performance levels up to hundred times better than a single CPU core[58,59]. Based on the time frames obtained in the benzamidine binding studies, nowadays it is possible to reconstruct those binding pathways of 5 to 10 ligands per week on a moderately sized cluster of 32-64 GPUs[50].

It can be expected that within next 20-25 years it will be possible to simulate all-atom dynamics of a small bacterium and describe the complete set of processes within[25]. Such simulations can be probably performed for only few milliseconds or even less and would not provide much information due to large timescales needed for diffusions of macromolecules in bacterial processes. However, simulation of the part of a cell or fundamental pathways in molecular biology, such as protein synthesis, would be possible on the several seconds time-scales in the nearest future. It is obvious that macromolecule-macromolecule interactions are essential for biological processes. One major task in MD simulations is to carry out large-scale simulations of protein-protein and protein-DNA interactions. MD simulations will address the binding pathways between the macromolecules and how the ligand binding sites created, at the interface or at allosteric sites? Simulations can be expected for very large complexes such as the nuclear pore. Based on generated trajectories from such simulations it will be possible to observe passage of the delivery protein as it transits through a nuclear pore. In addition, MD will impose significant impact on the proteomics since the protein folding will be possible for larger proteins. Based on the current state of the MD simulations extrapolation suggests that folding of the 300 residues protein (over 10 ms simulation of approximately 100 000 atoms) will be possible within 10 years. Folding of the larger proteins, consisting of multiple subunits and over 1000 residues, will be still challenging and likely be achievable within 25 years[25]. Significant improvement of the time-scales might come from omitting some motions in MD simulations. It might be possible to adequately sample conformational space without resolving all fast motions, thus reducing the computational power needed for the simulation of the particular system.

With constant gains in both computer power and algorithm design, MD simulation is expected to play an increasingly important role in the modern science from investigating the local motions in the biological macro-molecules to the development of novel pharmacological therapeutics. This methodology has emerged as an integral part of molecular biology studies, providing a fundamental and reliable tool for experimentalists.

REFERENCES

1   **Stout RD**, Herzenberg LA. The Fc receptor on thymus-derived lymphocytes. I. Detection of a subpopulation of murine T lymphocytes bearing the Fc receptor. *J Exp Med* 1975; **142**: 611-621

2   **Karplus M**, McCammon JA. Molecular dynamics simulations of biomolecules. *Nat Struct Biol* 2002; **9**: 646-652

3   **Sotomayor M**, Schulten K. Single-molecule experiments in vitro and in silico. *Science* 2007; **316**: 1144-1148

4   **Lee EH**, Hsin J, Sotomayor M, Comellas G, Schulten K. Discovery through the computational microscope. *Structure* 2009; **17**: 1295-1306

5   **McCammon JA**, Gelin BR, Karplus M. Dynamics of folded proteins. *Nature* 1977; **267**: 585-590

6   **Berger R**, Bernheim A. A comment on the paper: reciprocal translocation and the Philadelphia chromosome by Jessie L. Watt and Brenda M. Page, Hum. Genet. 42, 163--170 (1978) *Hum Genet* 1978; **44**: 357-358

7   **Hess B,** Kutzner C, van der Spoel D, Lindahl E. GROMACS 4: Algorithms for highly efficient, load-balanced, and scalable molecular simulation. *J Chem Theory Comput* 2008; **4**: 435-447

8   **Freddolino PL**, Liu F, Gruebele M, Schulten K. Ten-microsecond molecular dynamics simulation of a fast-folding WW domain. *Biophys J* 2008; **94**: L75-L77

9   **Power SG**, Challis JR. The effects of gestational age and intrafetal ACTH administration on the concentration of progesterone in the fetal membranes, endometrium, and myometrium of pregnant sheep. *Can J Physiol Pharmacol* 1987; **65**: 136-140

10 **Klepeis JL**, Lindorff-Larsen K, Dror RO, Shaw DE. Long-timescale molecular dynamics simulations of protein structure and function. *Curr Opin Struct Biol* 2009; **19**: 120-127

11 **Shaw DE.** Anton: A specialized machine for millisecond-scale molecular dynamics simulations of proteins. *Abstr Pap Am Chem S* 2009; **238**: 154-COMP

12 **Shaw DE**, Maragakis P, Lindorff-Larsen K, Piana S, Dror RO, Eastwood MP, Bank JA, Jumper JM, Salmon JK, Shan Y, Wriggers W. Atomic-level characterization of the structural dynamics of proteins. *Science* 2010; **330**: 341-346

13 **Wang W**, Donini O, Reyes CM, Kollman PA. Biomolecular simulations: recent developments in force fields, simulations of enzyme catalysis, protein-ligand, protein-protein, and protein-nucleic acid noncovalent interactions. *Annu Rev Biophys Biomol Struct* 2001; **30**: 211-243

14 **Warshel A**. Computer simulations of enzyme catalysis: methods, progress, and insights. *Annu Rev Biophys Biomol Struct* 2003; **32**: 425-443

15 **Gokey T**, Baird TT, Guliaev AB. Conformational dynamics of threonine 195 and the S1 subsite in functional trypsin variants. *J Mol Model* 2012; **18**: 4941-4954

16 **Bond PJ**, Sansom MS. The simulation approach to bacterial outer membrane proteins. *Mol Membr Biol* 2004; **21**: 151-161

17 **Zhu F**, Tajkhorshid E, Schulten K. Pressure-induced water transport in membrane channels studied by molecular dynamics. *Biophys J* 2002; **83**: 154-160

18 **Han BG**, Guliaev AB, Walian PJ, Jap BK. Water transport in AQP0 aquaporin: molecular dynamics studies. *J Mol Biol* 2006; **360**: 285-296

19 **Heninger GR**, Mueller PS, Davis LS. Depressive symptoms

and the glucose tolerance test and insulin tolerance test. *J Nerv Ment Dis* 1975; **161**: 421-432

20   **Almind G**, Bundgaard E, Larsen BS, Schoubye G. [Case finding by health visitors. Visits to 80-year-old persons living at home]. *Ugeskr Laeger* 1979; **141**: 465-469

21   **Stephens MK**. Handicapped children in Denmark. *Queens Nurs J* 1976; **19**: 193, 196

22   **Liu YH**, Wang XG, Wang QN, Qian MX, Wang RQ, Lu SZ, Liu J, Li GH, Chen YD. Efficacy of single oral doses of praziquantel in treatment of schistosoma japonicum infection. *Chin Med J* (Engl) 1986; **99**: 470-472

23   **Daggett V**. Protein folding-simulation. *Chem Rev* 2006; **106**: 1898-1916

24   **Wong CF**, McCammon JA. Protein flexibility and computer-aided drug design. *Annu Rev Pharmacol Toxicol* 2003; **43**: 31-45

25   **Borhani DW**, Shaw DE. The future of molecular dynamics simulations in drug discovery. *J Comput Aided Mol Des* 2012; **26**: 15-26

26   **Kruse AC**, Hu J, Pan AC, Arlow DH, Rosenbaum DM, Rosemond E, Green HF, Liu T, Chae PS, Dror RO, Shaw DE, Weis WI, Wess J, Kobilka BK. Structure and dynamics of the M3 muscarinic acetylcholine receptor. *Nature* 2012; **482**: 552-556

27   **Rosenbaum DM**, Zhang C, Lyons JA, Holl R, Aragao D, Arlow DH, Rasmussen SG, Choi HJ, Devree BT, Sunahara RK, Chae PS, Gellman SH, Dror RO, Shaw DE, Weis WI, Caffrey M, Gmeiner P, Kobilka BK. Structure and function of an irreversible agonist-β(2) adrenoceptor complex. *Nature* 2011; **469**: 236-240

28   **Brooks BR,** Bruccoleri RE, Olafson BD, States DJ, Swaminathan S, Karplus M. Charmm - a program for macromolecular energy, minimization, and dynamics calculations. *J Comput Chem* 1983; **4**: 187-217

29   **Karplus M**. Molecular dynamics of biological macromolecules: a brief history and perspective. *Biopolymers* 2003; **68**: 350-358

30   **Phillips JC**, Braun R, Wang W, Gumbart J, Tajkhorshid E, Villa E, Chipot C, Skeel RD, Kalé L, Schulten K. Scalable molecular dynamics with NAMD. *J Comput Chem* 2005; **26**: 1781-1802

31   **Pearlman DA,** Case DA, Caldwell JW, Ross WS, Cheatham TE, Debolt S, Ferguson D, Seibel G, Kollman P. Amber, a package of computer-programs for applying molecular mechanics, normal-mode analysis, molecular-dynamics and free-energy calculations to simulate the structural and energetic properties of molecules. *Comput Phys Commun* 1995; **91**: 1-41

32   **Levitt M**. Molecular dynamics of native protein. I. Computer simulation of trajectories. *J Mol Biol* 1983; **168**: 595-617

33   **Allinger NL,** Chen KS, Lii JH. An improved force field (MM4) for saturated hydrocarbons. *J Comput Chem* 1996; **17**: 642-668

34   **Beck DA**, Armen RS, Daggett V. Cutoff size need not strongly influence molecular dynamics results for solvated polypeptides. *Biochemistry* 2005; **44**: 609-616

35   **Darden T,** York D, Pedersen L. Particle Mesh Ewald - an N.Log(N) Method for Ewald Sums in Large Systems. *J Chem Phys* 1993; **98**: 10089-10092

36   **Adcock SA**, McCammon JA. Molecular dynamics: survey of methods for simulating the activity of proteins. *Chem Rev* 2006; **106**: 1589-1615

37   **Piana S**, Lindorff-Larsen K, Dirks RM, Salmon JK, Dror RO, Shaw DE. Evaluating the effects of cutoffs and treatment of long-range electrostatics in protein folding simulations. *PLoS One* 2012; **7**: e39918

38   **Sagui C**, Darden TA. Molecular dynamics simulations of biomolecules: long-range electrostatic effects. *Annu Rev Biophys Biomol Struct* 1999; **28**: 155-179

39   **Case DA**, Cheatham TE, Darden T, Gohlke H, Luo R, Merz KM, Onufriev A, Simmerling C, Wang B, Woods RJ. The Amber biomolecular simulation programs. *J Comput Chem* 2005; **26**: 1668-1688

40   **Cerutti DS**, Freddolino PL, Duke RE, Case DA. Simulations of a protein crystal with a high resolution X-ray structure: evaluation of force fields and water models. *J Phys Chem B* 2010; **114**: 12811-12824

41   **Chaudhury S**, Olson MA, Tawa G, Wallqvist A, Lee MS. Efficient Conformational Sampling in Explicit Solvent Using a Hybrid Replica Exchange Molecular Dynamics Method. *J Chem Theory Comput* 2012; **8**: 677-687

42   **Zhou R**. Replica exchange molecular dynamics method for protein folding simulation. *Methods Mol Biol* 2007; **350**: 205-223

43   **Dobson CM**. Protein folding and misfolding. *Nature* 2003; **426**: 884-890

44   **Anfinsen CB**. Principles that govern the folding of protein chains. *Science* 1973; **181**: 223-230

45   **Lindorff-Larsen K**, Piana S, Dror RO, Shaw DE. How fast-folding proteins fold. *Science* 2011; **334**: 517-520

46   **Settanni G**, Fersht AR. Downhill versus barrier-limited folding of BBL 3. Heterogeneity of the native state of the BBL peripheral subunit binding domain and its implications for folding mechanisms. *J Mol Biol* 2009; **387**: 993-1001

47   **Huang F**, Rajagopalan S, Settanni G, Marsh RJ, Armoogum DA, Nicolaou N, Bain AJ, Lerner E, Haas E, Ying L, Fersht AR. Multiple conformations of full-length p53 detected with single-molecule fluorescence resonance energy transfer. *Proc Natl Acad Sci USA* 2009; **106**: 20758-20763

48   **Liu YX,** Strumpfer J, Freddolino PL, Gruebele M, Schulten K. Structural characterization of lambda-repressor folding from all-atom molecular dynamics simulations. *J Phys Chem Lett* 2012; **3**: 1117-1123

49   **Durrant JD**, McCammon JA. Molecular dynamics simulations and drug discovery. *BMC Biol* 2011; **9**: 71

50   **Buch I**, Giorgino T, De Fabritiis G. Complete reconstruction of an enzyme-inhibitor binding process by molecular dynamics simulations. *Proc Natl Acad Sci USA* 2011; **108**: 10184-10189

51   **Dror RO,** Pan AC, Arlow DH, Borhani DW, Maragakis P, Shan YB, Xu HF, Shaw DE. Pathway and mechanism of drug binding to G-protein-coupled receptors. *P Natl Acad Sci USA* 2011; **108**: 13118-13123

52   **Rastelli G**, Del Rio A, Degliesposti G, Sgobba M. Fast and accurate predictions of binding free energies using MM-PBSA and MM-GBSA. *J Comput Chem* 2010; **31**: 797-810

53   **Brown SP**, Muchmore SW. Large-scale application of high-throughput molecular mechanics with Poisson-Boltzmann surface area for routine physics-based scoring of protein-ligand complexes. *J Med Chem* 2009; **52**: 3159-3165

54   **Whitford PC**, Geggier P, Altman RB, Blanchard SC, Onuchic JN, Sanbonmatsu KY. Accommodation of aminoacyl-tRNA into the ribosome involves reversible excursions along multiple pathways. *RNA* 2010; **16**: 1196-1204

55   **Arkhipov A**, Freddolino PL, Schulten K. Stability and dynamics of virus capsids described by coarse-grained modeling. *Structure* 2006; **14**: 1767-1777

56   **Robinson CV**, Sali A, Baumeister W. The molecular sociology of the cell. *Nature* 2007; **450**: 973-982

57   **Vendruscolo M**, Dobson CM. Protein dynamics: Moore's law in molecular biology. *Curr Biol* 2011; **21**: R68-R70

58   **Buch I**, Harvey MJ, Giorgino T, Anderson DP, De Fabritiis G. High-throughput all-atom molecular dynamics simulations using distributed computing. *J Chem Inf Model* 2010; **50**: 397-403

59   **Stone JE**, Hardy DJ, Ufimtsev IS, Schulten K. GPU-accelerated molecular modeling coming of age. *J Mol Graph Model* 2010; **29**: 116-125

Acknowledgments to reviewers of *World Journal of Methodology*

Events Calendar 2012

January 20-21, 2012
AGA Clinical Congress of Gastroen-
terology and Hepatology: Practice,
Evidence and Quality in 2012
Miami, FL, United States

January 26, 2012
Symposium of the Swiss Society
of Pharmacology and Toxicology,
Advances in Pharmacology - Car-
diovascular Pharmacology
Bern, Switzerland

January 28 – February 1, 2012
LabAutomation2012
Palm Springs Convention Center,
Palm Springs, CA, United States

February 1-4, 2012
3rd International Workshop on Med-
ical Image Analysis and Description
for Diagnosis System
Hotel Tivoli Victoria, Algarve, Por-
tugal

February 24, 2012
State Stem Cell Agency Governance
Subcommittee
San Francisco, CA, United States

March 11-14, 2012
Thoracic Imaging 2012
Hyatt Regency, Huntington Beach
Resort and Spa, Huntington Beach,
CA, United States

March 25-30, 2012
44th International Diagnostic Course

Davos (IDKD)
Davos, Switzerland

April 26-29, 2012
75th Anniversary of the Canadian
Association of Radiologists Annual
Scientific Meeting
Le Centre Sheraton, Montreal, Que-
bec, Canada

April 29 - May 4, 2012
EUCHEM Conference on Stereo-
chemistry
Brunnen, Switzerland

May 4-6, 2012
World Congress on Biotechnology
Hyderabad, India

June 7-9, 2012
ASCI 2012 - 6th Congress of Asian
Society of Cardiovascular Imaging
Bangkok, Thailand

June 9-13, 2012
2012 Annual Meeting of the Society
of Nuclear Medicine
Miami Beach, FL, United States

August 15-19, 2012
ICE 2012 - International Congress of
Endoscopy
Daegu, South Korea

October 28-30, 2012
EANM 2012 - Annual Congress of
the European Association of Nuclear
Medicine
Milan, Italy

World Journal of Methodology

INSTRUCTIONS TO AUTHORS

GENERAL INFORMATION

World Journal of Methodology (*World J Methodol*, *WJM*, online ISSN 2222-0682, DOI: 10.5662) is a bimonthly peer-reviewed, online, open-access (OA), journal supported by an editorial board consisting of 238 experts in methodology from 41 countries.

The biggest advantage of the OA model is that it provides free, full-text articles in PDF and other formats for experts and the public without registration, which eliminates the obstacle that traditional journals possess and usually delays the speed of the propagation and communication of scientific research results. The open access model has been proven to be a true approach that may achieve the ultimate goal of the journals, i.e. the maximization of the value to the readers, authors and society.

Maximization of personal benefits

The role of academic journals is to exhibit the scientific levels of a country, a university, a center, a department, and even a scientist, and build an important bridge for communication between scientists and the public. As we all know, the significance of the publication of scientific articles lies not only in disseminating and communicating innovative scientific achievements and academic views, as well as promoting the application of scientific achievements, but also in formally recognizing the "priority" and "copyright" of innovative achievements published, as well as evaluating research performance and academic levels. So, to realize these desired attributes of *WJM* and create a well-recognized journal, the following four types of personal benefits should be maximized. The maximization of personal benefits refers to the pursuit of the maximum personal benefits in a well-considered optimal manner without violation of the laws, ethical rules and the benefits of others. (1) Maximization of the benefits of editorial board members: The primary task of editorial board members is to give a peer review of an unpublished scientific article via online office system to evaluate its innovativeness, scientific and practical values and determine whether it should be published or not. During peer review, editorial board members can also obtain cutting-edge information in that field at first hand. As leaders in their field, they have priority to be invited to write articles and publish commentary articles. We will put peer reviewers' names and affiliations along with the article they reviewed in the journal to acknowledge their contribution; (2) Maximization of the benefits of authors: Since *WJM* is an open-access journal, readers around the world can immediately download and read, free of charge, high-quality, peer-reviewed articles from *WJM* official website, thereby realizing the goals and significance of the communication between authors and peers as well as public reading; (3) Maximization of the benefits of readers: Readers can read or use, free of charge, high-quality peer-reviewed articles without any limits, and cite the arguments, viewpoints, concepts, theories, methods, results, conclusion or facts and data of pertinent literature so as to validate the innovativeness, scientific and practical values of their own research achievements, thus ensuring that their articles have novel arguments or viewpoints, solid evidence and correct conclusion; and (4) Maximization of the benefits of employees: It is an iron law that a first-class journal is unable to exist without first-class editors, and only first-class editors can create a first-class academic journal. We insist on strengthening our team cultivation and construction so that every employee, in an open, fair and transparent environment, could contribute their wisdom to edit and publish high-quality ar-

ticles, thereby realizing the maximization of the personal benefits of editorial board members, authors and readers, and yielding the greatest social and economic benefits.

Aims and scope

WJM aims to rapidly report the most recent results in medical diagnostics, therapeutic techniques and equipment, clinical medical research, clinical and experimental techniques and methodology. It provides a platform to facilitate the integration of clinical medicine and experimental techniques and methodology to help clinicians improve diagnostic accuracy and therapeutic efficacy. The journal publishes original articles and reviews on the following topics: (1) Clinical medical techniques, including but not limited to those for pharmaceutical medicine, laboratory medicine, radioactive medicine, medical imaging, nuclear medicine, physical therapy, pathology, surgery, disinfection, nutritional therapy, transfusion and medical equipment; (2) Clinical medical research on etiology, epidemiology, pathogenesis, morphology and function, signs and symptoms, clinical trials, and evidence-based medicine; and (3) Laboratory methodology, including but not limited to techniques in DNA/RNA sequencing, preparation and transformation of competent cells, PCR, protein biochemistry, cell biology, genetics and epigenetics, immunology, microbiology, animal models of human pathologies, bioinformatics, and laboratory equipment manipulation and control.

Columns

The columns in the issues of *WJM* will include: (1) Editorial: To introduce and comment on major advances and developments in the field; (2) Frontier: To review representative achievements, comment on the state of current research, and propose directions for future research; (3) Topic Highlight: This column consists of three formats, including (A) 10 invited review articles on a hot topic, (B) a commentary on common issues of this hot topic, and (C) a commentary on the 10 individual articles; (4) Observation: To update the development of old and new questions, highlight unsolved problems, and provide strategies on how to solve the questions; (5) Guidelines for Basic Research: To provide Guidelines for basic research; (6) Guidelines for Clinical Practice: To provide guidelines for clinical diagnosis and treatment; (7) Review: To review systemically progress and unresolved problems in the field, comment on the state of current research, and make suggestions for future work; (8) Original Articles: To report innovative and original findings in basic and clinical medical research methodology; (9) Brief Articles: To briefly report the novel and innovative findings in basic and clinical medical research methodology; (10) Case Report: To report a rare or typical case; (11) Letters to the Editor: To discuss and make reply to the contributions published in *WJM*, or to introduce and comment on a controversial issue of general interest; (12) Book Reviews: To introduce and comment on quality monographs of basic and clinical medical research methodology; (13) Guidelines: To introduce Consensuses and Guidelines reached by international and national academic authorities worldwide on the research basic and clinical medical research methodology; and (14) Voices: to publicize methodology-related communications that have been rejected or impossible for publication elsewhere due to evident prejudice and/or unreasonable reasons. Similarly, your experiences of the proven mistreatment during the past grant applications can be narrated or documented in this corner. The corresponding responses and echoes from readers are also welcome here.

Instructions to authors

SPECIAL STATEMENT

All articles published in this journal represent the viewpoints of the authors except where indicated otherwise.

Biostatistical editing
Statistical review is performed after peer review. We invite an expert in Biomedical Statistics to evaluate the statistical method used in the paper, including *t*-test (group or paired comparisons), chi-squared test, Ridit, probit, logit, regression (linear, curvilinear, or stepwise), correlation, analysis of variance, analysis of covariance, *etc*. The reviewing points include: (1) Statistical methods should be described when they are used to verify the results; (2) Whether the statistical techniques are suitable or correct; (3) Only homogeneous data can be averaged. Standard deviations are preferred to standard errors. Give the number of observations and subjects (*n*). Losses in observations, such as drop-outs from the study should be reported; (4) Values such as ED50, LD50, IC50 should have their 95% confidence limits calculated and compared by weighted probit analysis (Bliss and Finney); and (5) The word 'significantly' should be replaced by its synonyms (if it indicates extent) or the *P* value (if it indicates statistical significance).

Conflict-of-interest statement
In the interests of transparency and to help reviewers assess any potential bias, *WJM* requires authors of all papers to declare any competing commercial, personal, political, intellectual, or religious interests in relation to the submitted work. Referees are also asked to indicate any potential conflict they might have reviewing a particular paper. Before submitting, authors are suggested to read "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Ethical Considerations in the Conduct and Reporting of Research: Conflicts of Interest" from International Committee of Medical Journal Editors (ICMJE), which is available at: http://www.icmje.org/ethical_4conflicts.html.

Sample wording: [Name of individual] has received fees for serving as a speaker, a consultant and an advisory board member for [names of organizations], and has received research funding from [names of organization]. [Name of individual] is an employee of [name of organization]. [Name of individual] owns stocks and shares in [name of organization]. [Name of individual] owns patent [patent identification and brief description].

Statement of informed consent
Manuscripts should contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee or it should be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted. Authors should also draw attention to the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964, as revised in 2004).

Statement of human and animal rights
When reporting the results from experiments, authors should follow the highest standards and the trial should conform to Good Clinical Practice (for example, US Food and Drug Administration Good Clinical Practice in FDA-Regulated Clinical Trials; UK Medicines Research Council Guidelines for Good Clinical Practice in Clinical Trials) and/or the World Medical Association Declaration of Helsinki. Generally, we suggest authors follow the lead investigator's national standard. If doubt exists whether the research was conducted in accordance with the above standards, the authors must explain the rationale for their approach and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study.

Before submitting, authors should make their study approved by the relevant research ethics committee or institutional review board. If human participants were involved, manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and appropriate informed consent of each. Any personal item or information will not be published without explicit consents from the involved patients. If experimental animals were used, the materials and methods (experimental procedures) section must clearly indicate that appropriate measures were taken to minimize pain or discomfort, and details of animal care should be provided.

SUBMISSION OF MANUSCRIPTS

Manuscripts should be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Number all pages consecutively, and start each of the following sections on a new page: Title Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References, Tables, Figures, and Figure Legends. Neither the editors nor the publisher are responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of Baishideng Publishing Group Co., Limited, and may not be reproduced by any means, in whole or in part, without the written permission of both the authors and the publisher. We reserve the right to copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory animals of their institution or national animal welfare committee. For the sake of transparency in regard to the performance and reporting of clinical trials, we endorse the policy of the ICMJE to refuse to publish papers on clinical trial results if the trial was not recorded in a publicly-accessible registry at its outset. The only register now available, to our knowledge, is http://www.clinicaltrials.gov sponsored by the United States National Library of Medicine and we encourage all potential contributors to register with it. However, in the case that other registers become available you will be duly notified. A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

Authors should retain one copy of the text, tables, photographs and illustrations because rejected manuscripts will not be returned to the author(s) and the editors will not be responsible for loss or damage to photographs and illustrations sustained during mailing.

Online submissions
Manuscripts should be submitted through the Online Submission System at: http://www.wjgnet.com/esps/. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS

(http://www.wjgnet.com/2222-0682/g_info_20100722180909. htm) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to wjm@wjgnet.com, or by telephone: +86-10-85381892. If you submit your manuscript online, do not make a postal contribution. Repeated online submission for the same manuscript is strictly prohibited.

MANUSCRIPT PREPARATION

All contributions should be written in English. All articles must be submitted using word-processing software. All submissions must be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Style should conform to our house format. Required information for each of the manuscript sections is as follows:

Title page
Title: Title should be less than 12 words.

Running title: A short running title of less than 6 words should be provided.

Authorship: Authorship credit should be in accordance with the standard proposed by ICMJE, based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.

Institution: Author names should be given first, then the complete name of institution, city, province and postcode. For example, Xu-Chen Zhang, Li-Xin Mei, Department of Pathology, Chengde Medical College, Chengde 067000, Hebei Province, China. One author may be represented from two institutions, for example, George Sgourakis, Department of General, Visceral, and Transplantation Surgery, Essen 45122, Germany; George Sgourakis, 2nd Surgical Department, Korgialenio-Benakio Red Cross Hospital, Athens 15451, Greece

Author contributions: The format of this section should be: Author contributions: Wang CL and Liang L contributed equally to this work; Wang CL, Liang L, Fu JF, Zou CC, Hong F and Wu XM designed the research; Wang CL, Zou CC, Hong F and Wu XM performed the research; Xue JZ and Lu JR contributed new reagents/analytic tools; Wang CL, Liang L and Fu JF analyzed the data; and Wang CL, Liang L and Fu JF wrote the paper.

Supportive foundations: The complete name and number of supportive foundations should be provided, e.g. Supported by National Natural Science Foundation of China, No. 30224801

Correspondence to: Only one corresponding address should be provided. Author names should be given first, then author title, affiliation, the complete name of institution, city, postcode, province, country, and email. All the letters in the email should be in lower case. A space interval should be inserted between country name and email address. For example, Montgomery Bissell, MD, Professor of Medicine, Chief, Liver Center, Gastroenterology Division, University of California, Box 0538, San Francisco, CA 94143, United States. montgomery.bissell@ucsf.edu

Telephone and fax: Telephone and fax should consist of +, country number, district number and telephone or fax number, e.g. Telephone: +86-10-85381892 Fax: +86-10-85381893

Peer reviewers: All articles received are subject to peer review. Normally, three experts are invited for each article. Decision for acceptance is made only when at least two experts recommend an article for publication. Reviewers for accepted manuscripts are acknowledged in each manuscript, and reviewers of articles which

were not accepted will be acknowledged at the end of each issue. To ensure the quality of the articles published in *WJM*, reviewers of accepted manuscripts will be announced by publishing the name, title/position and institution of the reviewer in the footnote accompanying the printed article. For example, reviewers: Professor Jing-Yuan Fang, Shanghai Institute of Digestive Disease, Shanghai, Affiliated Renji Hospital, Medical Faculty, Shanghai Jiaotong University, Shanghai, China; Professor Xin-Wei Han, Department of Radiology, The First Affiliated Hospital, Zhengzhou University, Zhengzhou, Henan Province, China; and Professor Anren Kuang, Department of Nuclear Medicine, Huaxi Hospital, Sichuan University, Chengdu, Sichuan Province, China.

Abstract
There are unstructured abstracts (no less than 256 words) and structured abstracts (no less than 480). The specific requirements for structured abstracts are as follows:

An informative, structured abstracts of no less than 480 words should accompany each manuscript. Abstracts for original contributions should be structured into the following sections. AIM (no more than 20 words): Only the purpose should be included. Please write the aim as the form of "To investigate/study/…; MATERIALS AND METHODS (no less than 140 words); RESULTS (no less than 294 words): You should present P values where appropriate and must provide relevant data to illustrate how they were obtained, e.g. 6.92 ± 3.86 vs 3.61 ± 1.67, $P < 0.001$; CONCLUSION (no more than 26 words).

Key words
Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

Text
For articles of these sections, original articles and brief articles, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both. The main text format of these sections, editorial, topic highlight, case report, letters to the editors, can be found at: http://www.wjgnet.com/2222-0682/g_info_20100725072755.htm.

Illustrations
Figures should be numbered as 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. Detailed legends should not be provided under the figures. This part should be added into the text where the figures are applicable. Figures should be either Photoshop or Illustrator files (in tiff, eps, jpeg formats) at high-resolution. Examples can be found at: http://www.wjgnet.com/1007-9327/13/4520. pdf; http://www.wjgnet.com/1007-9327/13/4554.pdf; http://www.wjgnet.com/1007-9327/13/4891.pdf; http://www.wjgnet.com/1007-9327/13/4986.pdf; http://www.wjgnet.com/1007-9327/13/4498.pdf. Keeping all elements compiled is necessary in line-art image. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. File names should identify the figure and panel. Avoid layering type directly over shaded or textured areas. Please use uniform legends for the same subjects. For example: Figure 1 Pathological changes in atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: …*etc*. It is our principle to publish high resolution-figures for the printed and E-versions.

Tables
Three-line tables should be numbered 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each table. Detailed legends should not be included under tables, but rather added into the text where applicable. The information should complement, but not duplicate the text. Use one horizontal line under the title, a second under column heads, and a third below the Table, above any

footnotes. Vertical and italic lines should be omitted.

Notes in tables and illustrations

Data that are not statistically significant should not be noted. $^aP < 0.05$, $^bP < 0.01$ should be noted ($P > 0.05$ should not be noted). If there are other series of P values, $^cP < 0.05$ and $^dP < 0.01$ are used. A third series of P values can be expressed as $^eP < 0.05$ and $^fP < 0.01$. Other notes in tables or under illustrations should be expressed as ^{1}F, ^{2}F, ^{3}F; or sometimes as other symbols with a superscript (Arabic numerals) in the upper left corner. In a multi-curve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, *etc.*, in a certain sequence.

Acknowledgments

Brief acknowledgments of persons who have made genuine contributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

REFERENCES

Coding system

The author should number the references in Arabic numerals according to the citation order in the text. Put reference numbers in square brackets in superscript at the end of citation content or after the cited author's name. For citation content which is part of the narration, the coding number and square brackets should be typeset normally. For example, "Crohn's disease (CD) is associated with increased intestinal permeability[1,2]". If references are cited directly in the text, they should be put together within the text, for example, "From references[19,22-24], we know that..."

When the authors write the references, please ensure that the order in text is the same as in the references section, and also ensure the spelling accuracy of the first author's name. Do not list the same citation twice.

PMID and DOI

Pleased provide PubMed citation numbers to the reference list, e.g. PMID and DOI, which can be found at http://www.ncbi.nlm.nih. gov/sites/entrez?db=pubmed and http://www.crossref.org/SimpleTextQuery/, respectively. The numbers will be used in E-version of this journal.

Style for journal references

Authors: the name of the first author should be typed in bold-faced letters. The family name of all authors should be typed with the initial letter capitalized, followed by their abbreviated first and middle initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR). The title of the cited article and italicized journal title (journal title should be in its abbreviated form as shown in PubMed), publication date, volume number (in black), start page, and end page [PMID: 11819634 DOI: 10.3748/wjg.13.5396].

Style for book references

Authors: the name of the first author should be typed in bold-faced letters. The surname of all authors should be typed with the initial letter capitalized, followed by their abbreviated middle and first initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR) Book title. Publication number. Publication place: Publication press, Year: start page and end page.

Format
Journals
English journal article (list all authors and include the PMID where applicable)

1   **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13. 6356]

Chinese journal article (list all authors and include the PMID where applicable)

2   **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3   **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4   **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494. 09]

Both personal authors and an organization as author

5   **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju. 0000067940.76090.73]

No author given

6   21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325. 7357.184]

Volume with supplement

7   **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/ j.1526-4610.42.s2.7.x]

Issue with no volume

8   **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.10 97/00003086-200208000-00026]

No volume or issue

9   Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books
Personal author(s)

10   **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11   **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12   **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13   **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14   **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15   Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ ncidod/eid/index.htm

Patent (list all authors)

16   **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 2002103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express t test as t (in italics), F test as F (in italics), chi square test as χ^2 (in Greek), related coefficient as r (in italics), degree of freedom as υ (in Greek), sample number as n (in italics), and probability as P (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 µg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc*. Arabic numerals such as 23, 243, 641 should be read 23 243 641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/2222-0682/g_info_20100725073806.htm.

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: t time or temperature, c concentration, A area, l length, m mass, V volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc*.
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc*.
Biology: *H. pylori*, *E coli*, *etc*.

Examples for paper writing

Editorial: http://www.wjgnet.com/2222-0682/g_info_20100725071851.htm

Frontier: http://www.wjgnet.com/2222-0682/g_info_20100725071932.htm

Topic highlight: http://www.wjgnet.com/2222-0682/g_info_20100725072121.htm

Observation: http://www.wjgnet.com/2222-0682/g_info_20100725072232.htm

Guidelines for basic research: http://www.wjgnet.com/2222-0682/g_info_20100725072344.htm

Guidelines for clinical practice: http://www.wjgnet.com/2222-0682/g_info_20100725072543.htm

Review: http://www.wjgnet.com/2222-0682/g_info_20100725072656.htm

Original articles: http://www.wjgnet.com/2222-0682/g_info_20100725072755.htm

Brief articles: http://www.wjgnet.com/2222-0682/g_info_20100725072920.htm

Case report: http://www.wjgnet.com/2222-0682/g_info_20100725073015.htm

Letters to the editor: http://www.wjgnet.com/2222-0682/g_info_20100725073136.htm

Book reviews: http://www.wjgnet.com/2222-0682/g_info_20100725073214.htm

Guidelines: http://www.wjgnet.com/2222-0682/g_info_20100725073300.htm

SUBMISSION OF THE REVISED MANUSCRIPTS AFTER ACCEPTED

Authors must revise their manuscript carefully according to the revision policies of Baishideng Publishing Group Co., Limited. The revised version, along with the signed copyright transfer agreement, responses to the reviewers, and English language Grade B certificate (for non-native speakers of English), should be submitted to the online system via the link contained in the e-mail sent by the editor. If you have any questions about the revision, please send e-mail to esps@wjgnet.com.

Language evaluation

The language of a manuscript will be graded before it is sent for revision. (1) Grade A: priority publishing; (2) Grade B: minor language polishing; (3) Grade C: a great deal of language polishing needed; and (4) Grade D: rejected. Revised articles should reach Grade A or B.

Copyright assignment form

Please download a Copyright assignment form from http://www.wjgnet.com/2222-0682/g_info_20100725073726.htm.

Responses to reviewers

Please revise your article according to the comments/suggestions provided by the reviewers. The format for responses to the reviewers' comments can be found at: http://www.wjgnet.com/2222-0682/g_info_20100725073445.htm.

Proof of financial support

For paper supported by a foundation, authors should provide a copy of the document and serial number of the foundation.

Links to documents related to the manuscript

WJM will be initiating a platform to promote dynamic interactions between the editors, peer reviewers, readers and authors. After a manuscript is published online, links to the PDF version of the submitted manuscript, the peer-reviewers' report and the revised manuscript will be put on-line. Readers can make comments on the peer reviewer's report, authors' responses to peer reviewers, and the revised manuscript. We hope that authors will benefit from this feedback and be able to revise the manuscript accordingly in a timely manner.

Science news releases

Authors of accepted manuscripts are suggested to write a science news item to promote their articles. The news will be released rapidly at EurekAlert/AAAS (http://www.eurekalert.org). The title for news items should be less than 90 characters; the summary should be less than 75 words; and main body less than 500 words. Science news items should be lawful, ethical, and strictly based on your original content with an attractive title and interesting pictures.

Publication fee

WJM is an international, peer-reviewed, OA, online journal. Articles published by this journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license. Authors of accepted articles must pay a publication fee. Publication fee: 600 USD per article. Editorial, topic highlights, book reviews and letters to the editor are published free of charge.