

Molecular docking analysis of an isoflavone derivative with the protein phosphatase 1 from *Leishmania donovani*

Rahila Qureshi, Malini Devi Alaparthy, Prathyusha Sai Eligati, Syed Rizwan Hasan Razvi, Komal Paresh Walvekar, Mohammad Afraa & Someswar Rao Sagurthi*

Drug Design & Molecular Medicine Laboratory, Department of Genetics & Biotechnology, Osmania University, Hyderabad, Telangana-500007, India; *Corresponding author; Someswar Rao Sagurthi – E-mail: drsomeswar@osmania.ac.in

Received September 21, 2020; Revised October 24, 2020; Accepted October 24, 2020; Published November 30, 2020

DOI: 10.6026/97320630016942

The authors are responsible for the content of this article. The Editorial and the publisher has taken reasonable steps to check the content of the article in accordance to publishing ethics with adequate peer reviews deposited at PUBLONS.

Declaration on official E-mail:

The corresponding author declares that official e-mail from their institution is not available for all authors

Declaration on Publication Ethics:

The authors state that they adhere with COPE guidelines on publishing ethics as described elsewhere at <https://publicationethics.org/>. The authors also undertake that they are not associated with any other third party (governmental or non-governmental agencies) linking with any form of unethical issues connecting to this publication. The authors also declare that they are not withholding any information that is misleading to the publisher in regard to this article.

Abstract:

Leishmaniasis is one of the most neglected diseases with high morbidity and mortality rate. Severe side effects with existing drug and lack of proper vaccine encouraged us to design alternative models to combat the disease. We showed that PP1 of *Leishmania donovani* mediates immunomodulation in host macrophages needed for parasite survival. Therefore, it is of interest to report the molecular docking analysis of 512 isoflavone derivatives with the phosphatase 1 protein from *Leishmania donovani* to highlight compound 362 (5-hydroxy-5-(9-[2-methoxy-2-(2-methylfuran-3-yl) ethyl]-1H, 3H, 4H, 10bH-pyrano[4,3-c]chromen-3-yl)pentanoic acid) having good binding features and acceptable ADMET properties for further consideration.

Key Words: *Leishmania donovani*; Resistance; Protein phosphatase 1; Isoflavonoids; Docking.

Background:

Leishmaniasis is a vector borne disease, caused by protozoan parasites of the genus *Leishmania*. This disease is recognized as a neglected tropical disease and mainly associated with poverty stricken areas with endemicity in more than 98 countries. Approximately 350 million people are estimated to be at risk of leishmaniasis, with an estimated 0.7 to 1 million new cases occurring annually [1]. The severity of the disease varies from

disfiguring cutaneous form to the more fatal and life threatening visceral leishmaniasis. *Leishmania donovani* causes visceral form and is transmitted by phlebotomine fly to the human host. The flagellated promastigotes differentiate into non-flagellated and pathogenic amastigotes after being phagocytized by macrophages. Non-availability of vaccine and limitations of chemotherapy as well as toxicity and emergence of resistance further aggravate the condition and press for the need of exploring important leishmanial

proteins inhibitors which can also be exploited further for the development of antileishmanials [2]. To survive and differentiate in the hostile conditions inside the macrophage, a stress response through signal transduction gets initiated in the parasite [3]. Reversible phosphorylation through kinases and phosphatases is the critical event during the parasitic stress response and known to cause alterations in expression, interaction and activity profiles of various proteins [3]. The phosphatome and kinome analysis of various parasites from trypanosomatid family and species of *Leishmania* highlights the abundance of protein phosphatases maintaining phosphorylation-dephosphorylation equilibrium. Protein phosphatases dephosphorylate substrates and play an important role in posttranslational modifications [4], cellular differentiation [5] and drug resistance [6]. Almost 96–99% proteins in the eukaryotes proteome get phosphorylated on serine and threonine residues through ser/thr phosphatases [7]. PP1 group of ser/thr phosphatases is ubiquitously present in most of the cells and proven to be inhibited by known natural compounds such as okadaic acid, calyculin A, microcystin, tautomycin and cantharadin. Inhibition of PP1 in *P. falciparum* has resulted in hampered parasitic growth and its ablation through RNAi resulted in decreased DNA synthesis, confirming its role during replication of the parasite [8]. Additionally, calyculin A and okadaic acid mediated inhibition of PP1 advocated towards an important role played by PP1 during parasite's attachment to their host cells in *Trichomonas vaginalis* [9], *Toxoplasma gondii* [10] and *P. falciparum* [11]. Similarly, inhibition of PP1 in *T. brucei* with the same compound resulted in defected segregation of kinetoplasts, stalled cytokinesis and interrupted organellar cycle [12]. Another detailed study evaluated inhibition of PP1 through tautomycin and demonstrated the regulation of mitogen-activated protein kinase (MAPK) by interacting with Raf-1, hence act as a positive regulator of Raf-MEK-ERK pathway [13]. A small molecule like guanabenz and its derivative Sephin selectively inhibited the regulatory subunit of PP1 *in vivo*. Remarkably, Sephin1 does not inhibit the constitutively present form of PP1 regulatory subunit (PP1R15B) but specifically binds and inhibits the stress-induced form (PP1R15A) of the same and prevented diseases in mice related to protein misfolding [14]. Other mechanisms including blocking the PP1 regulatory subunit from accessing its physiological substrate or inducing disassembly of regulatory and catalytic subunits have also been suggested to achieve selective inhibition of PP1 holoenzyme [15]. Thus, owing to the indispensable role played by PP1, several inhibitors have been explored in higher eukaryotes including mice and human. However, in spite of being involved in many cellular processes, the fundamental mechanistic pertaining to the critical phosphatase inhibition is needed. Recently, we have characterized the PP1 of *L. donovani* (*LdPP1*) that shows protein-

mediated immunomodulation in human macrophages. The three-dimensional structure of *LdPP1*, determined by homology modelling, displayed all the characteristic features corresponding to the known PP1 structures. Binding of known inhibitor (okadaic acid) with *LdPP1* has provided insights into the molecular mechanism of inhibition [16]. The isoflavones and their derivatives have been proven as leishmanicidal and anti-plasmodial activity [17]. Therefore, it is of interest to report the molecular docking analysis of 512 isoflavone derivatives with the phosphatase 1 protein from *Leishmania donovani* to highlight compound 362 (5-hydroxy-5-(9-[2-methoxy-2-(2-methylfuran-3-yl)ethyl]-1H, 3H, 4H, 10bH-pyrano[4,3-c]chromen-3-yl}pentanoic acid) having acceptable ADMET properties for further consideration.

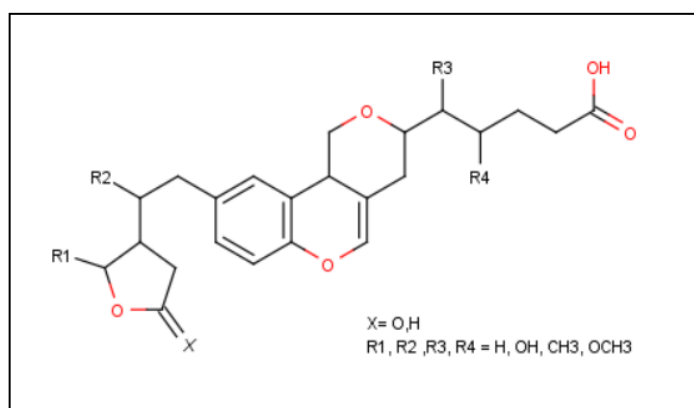


Figure 1: Basic scaffold of designed molecule with different substituents.

Methodology:

Compound dataset:

Isoflavonoids and their derivatives have been evidenced the multiple uses with low toxicity profile. Okadaic acid is a toxin produced by dinoflagellates, which inhibits human PP1. Combining the molecular binding profile of the two molecules we rationally designed a library of 512 molecules against *LdPP1* by using MarvinSketch 5.6.0.2. The pharmacological efficiency of these molecules is further validated by okadaic acid, known inhibitor of protein phosphatases produced from sponges and shellfish.

Protein homology modelling:

The structural characterization of *LdPP1* has been reported earlier [16]. Briefly, homology modelling of the protein was performed using Modeller [18]. This generated five different models, of which best model with better stereo chemical properties was chosen

through ModLoop [19]. Finally, the energy minimized structure required for docking was developed using the GROMACS program.

Ligand and protein preparation:

All the designed molecules were cleaned and converted to three-dimensional form with explicit hydrogen bonds by using MarvinView application 5.6.0.2. Further, the molecules were saved in .pdb format for docking studies. Simultaneously, the protein was also prepared by assigning missing bonds and bond orders. Also, charges were assigned at necessary places and the final structure was saved in .pdb format.

Molecular docking of Compounds:

Molecular docking provides information regarding how the ligand interacts with target protein at molecular level. In the present study, we used Molegro Virtual Docker 2010.4.0 to screen the compound dataset at docking site [20-23]. A mathematical notation called Moldock score, which is based on Piecewise Linear Potential

(PLP), gave the protein-ligand interaction. The total Moldock score represents the sum of total internal ligand energies and protein interaction energies along with soft penalties. Accordingly, the compound with highest Moldock score has highest binding energies with good protein-ligand interactions.

Table 1: Prediction and analysis of pockets and descriptors of LdPP1 by using DoGSiteScorer.

Cavity No.	Volume [Å ³]	Surface [Å ²]	Depth [Å]	Simple Score
P0	532.86	886.69	14.76	0.34
P1	424.77	508.1	17.59	0.23
P2	394.94	582.87	21.6	0.19
P3	326.4	410.69	16.25	0.13
P4	259.78	362.6	11.39	0.08
P5	229.06	300.06	16.46	0

Table 2: Lead compounds showing comparable affinity (Rerank score) towards LdPP1

Ligand	MolDock Score	Rerank Score	HBond
331.mol	-136.68	-101.04	-10.19
362.mol	-148.38	-112.17	-8.34
467.mol	-137.43	-103.22	-6.86
Okadaic acid	-141.45	-85.86	-6

Table 3: Table showing ADMET properties of screened compounds by ADMETS

Energy overview: Descriptors	Value	MolDock Score	Rerank Weight	Rerank Score
Total Energy		-148.387		-112.176
External Ligand interactions		-150.237		-125.003
Protein - Ligand interactions		-150.237		-125.003
Steric (by PLP)	-131.05	-131.052	0.686	-89.902
Steric (by LJ12-6)	-37.348		0.533	-19.906
Hydrogen bonds	-19.185	-19.185	0.792	-15.194
Hydrogen bonds (no directionality)	-21.331			0
Electrostatic (short range)	0	0	0.892	0
Electrostatic (long range)	0	0	0.156	0
Internal Ligand interactions		1.85		12.827
Torsional strain	1.108	1.108	0.938	1.039
Torsional strain (sp2-sp2)		0	0.636	0
Hydrogen bonds	0			0
Steric (by PLP)	0.742	0.742	0.172	0.128
Steric (by LJ12-6)	83.888		0.139	11.666
Electrostatic	0	0	0.437	0

Table 4: Table showing ADMET properties of screened compounds by ADMETSAR.

	331		362		467		Okadaic acid	
Absorption								
Blood-Brain Barrier	BBB+	0.61	BBB+	0.67	BBB+	0.6	BBB+	0.75
Human Intestinal Absorption	HIA+	0.89	HIA+	0.91	HIA+	0.9	HIA+	0.77
Caco-2 Permeability	Caco2-	0.73	Caco2-	0.55	Caco2-	0.66	Caco2-	0.74
P-glycoprotein Substrate	Substrate	0.81	Substrate	0.82	Substrate	0.84	Substrate	0.84
P-glycoprotein Inhibitor	Non-inhibitor	0.85	Non-inhibitor	0.59	Non-inhibitor	0.59	Non-inhibitor	0.58
Renal Organic Cation Transporter Distribution & Metabolism	Non-inhibitor	0.82	Non-inhibitor	0.69	Non-inhibitor	0.73	Non-inhibitor	0.8
CYP450 2C9 Substrate	Non-substrate	0.86	Non-substrate	0.87	Non-substrate	0.84	Non-substrate	0.88
CYP450 2D6 Inhibitor	Non-substrate	0.85	Non-substrate	0.84	Non-substrate	0.86	Non-substrate	0.89
CYP450 3A4 Substrate	Substrate	0.67	Substrate	0.69	Substrate	0.73	Substrate	0.73
CYP450 1A2 Inhibitor	Inhibitor	0.53	Inhibitor	0.57	Inhibitor	0.7	Non-inhibitor	0.86
CYP450 2C9 Inhibitor	Non-inhibitor	0.76	Non-inhibitor	0.81	Non-inhibitor	0.86	Non-inhibitor	0.9
CYP450 2D6 Inhibitor	Non-inhibitor	0.84	Non-inhibitor	0.77	Non-inhibitor	0.87	Non-inhibitor	0.95

CYP450 2C19 Inhibitor	Non-inhibitor	0.74	Non-inhibitor	0.7	Non-inhibitor	0.87	Non-inhibitor	0.89
CYP450 3A4 Inhibitor	Non-inhibitor	0.5	Non-inhibitor	0.6	Inhibitor	0.67	Non-inhibitor	0.81
CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	0.9	Low CYP Inhibitory Promiscuity	0.52	Low CYP Inhibitory Promiscuity	0.73	Low CYP Inhibitory Promiscuity	0.96
Excretion & Toxicity								
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.82	Weak inhibitor	0.88	Weak inhibitor	0.73	Weak inhibitor	0.94
AMES Toxicity	Non AMES toxic	0.75	Non AMES toxic	0.66	AMES toxic	0.6	Non AMES toxic	0.91
Carcinogens	Non-carcinogens	0.97	Non-carcinogens	0.96	Non-carcinogens	0.96	Non-carcinogens	0.97

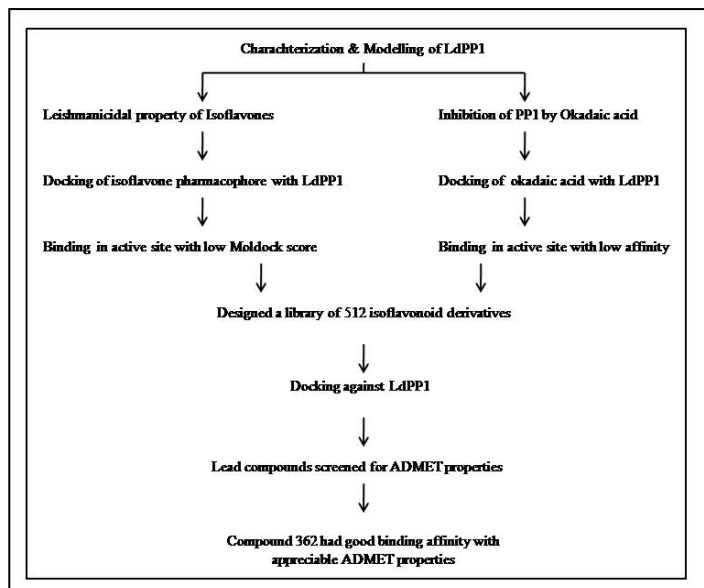


Figure 2: Flow chart outlining the steps in optimizing the lead molecule against *LdPP1*

ADMET profiling of compounds:

The druggable nature of the compounds cannot be predicted by ligand protein interactions alone. It should also possess good pharmacokinetic properties with low toxicity profile. Thus, complete ADMET profiling of the compounds was performed using ADMETSAR webserver.

Results & Discussion:

Basic scaffold of the designed molecules along with different substituents at R1, R2, R3 and R4 was shown in the **Figure 1**. In the library (**Table 1**), few molecules have shown best affinity towards *LdPP1*, out of which top three molecules along with okadaic acid structures were given in the **Table 2**. All the molecules have shown almost equal affinity towards *LdPP1* as okadaic acid at the active site. Compound 362 has exhibited highest affinity with Moldock score of -148.38 and Rerank score -112.17 which is comparable to

okadaic acid with comparable Moldock -141.45 and least rerank score as -85.86 (**Table 2**). The docking profile of compound 362, with energy descriptor values and their interactions in detail were given in **Table 3**. In external ligand interactions, which include mainly protein-ligand interactions, the steric interactions provide major stability with value -131.05 based on piecewise linear potential. Along with, hydrogen bond interactions also play a key role in stabilizing the ligand protein duo. Optimal affinity of the molecules towards *LdPP1* was contributed by different interactions namely van der Waals forces, conventional hydrogen bond, carbon hydrogen bond, Pi-cationic and Pi-alkyl bonds. Asp59, Asp87, Arg91, Asn119, His120, Ile125, Tyr129, His168, Trp201, Arg217, Gly218, Val219, His244, Gln245, Val246, Phe263, Tyr268 are the amino acids having various interactions with target protein. The lead molecule 362, demonstrates van der Waals interactions with Asp87, Arg91, His120, Ile125, Tyr129, His168, Gly218, Val219, Gln245, Phe263 conventional carbon hydrogen interactions with Asp59, Val246, Tyr268, carbon hydrogen bond with Asn119, Trp201, His244 and pi-cationic interaction with Arg217 (**Figure 4**). The lead molecules with highest affinity were assessed for their ADMET properties, and the result demonstrated well appreciable pharmacokinetic parameters i.e. absorption, distribution, metabolism and excretion as shown in **Table 4**. All the compounds exhibited non-carcinogenic properties, which are comparable to okadaic acid. **Figure 2** illustrates the steps involved in optimizing the lead molecule against *LdPP1*. The high affinity of isoflavone derivatives towards *LdPP1* envisage a novel class of inhibitors in the treatment of leishmanial infections with low toxicity profile and druggable nature of compounds might support further research in this area to subside the leishmanial infection rate.

Conclusion:

We report binding structure feature data on compound 362 (5-hydroxy-5-{9-[2-methoxy-2-(2-methylfuran-3-yl)ethyl]-1H, 3H, 4H, 10bH-pyrano [4,3-c] chromen-3-yl}pentanoic acid) with *PP1* having acceptable ADMET properties for further consideration to combat the disease Leishmaniasis.

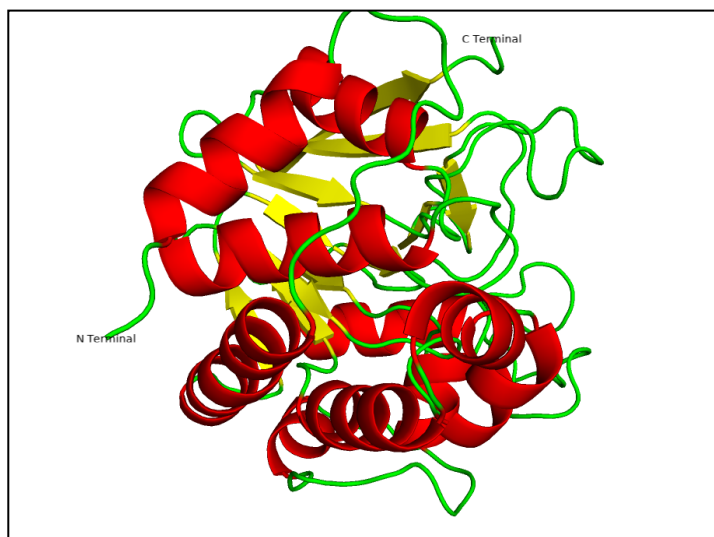


Figure 3: 3D homology model structure of *Ldpp1*

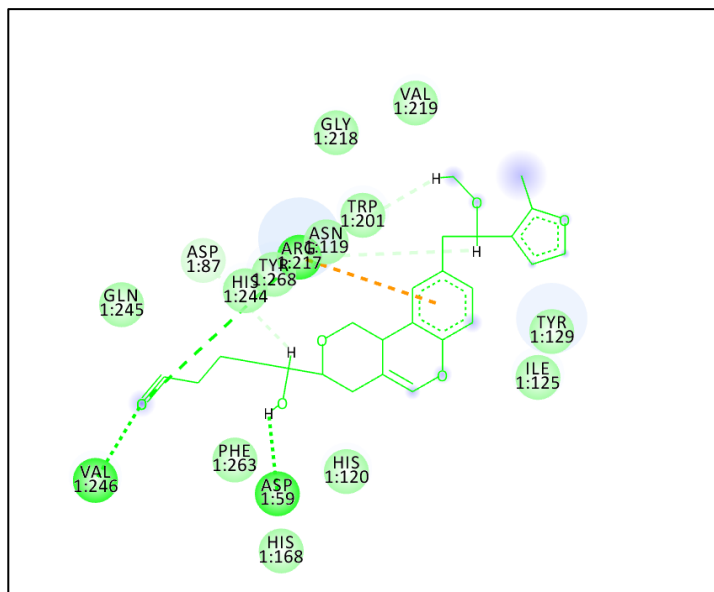


Figure 4: 2D representation of ligand-receptor with *LdPP1*. Orange colour dotted line represents Pi-cation interaction; Light green colour thick dotted line represents conventional hydrogen bonds, whereas the light green colour thin dotted line represents carbon hydrogen bonds. The non-bonded free amino acids around shows van der Waals interactions.

Acknowledgement:

The authors thank Dept. of Genetics & Biotechnology and Central Facilities for Research and Development (CFRD), Osmania University, Hyderabad for providing lab-facilities.

References:

- [1] <https://www.who.int/news-room/factsheets/detail/leishmaniasis>
- [2] Vijayakumar S *et al. Acta tropica*. 2018 **181**:95 [PMID: 29452111]
- [3] Hunter *et al. Cell* 2000 **100**:113. [PMID: 10647936]
- [4] Morales MA *et al. Proc Natl AcadSci U S A*. 2010 **107**:8381. [PMID: 20404152]
- [5] Szoor B *et al. PLoSPathog*. 2013 **9**:e1003689. [PMID: 24146622]
- [6] Bhandari V *et al. Chemother*. 2014 **58**:2580. [PMID: 24550335]
- [7] Bradshaw N *et al. Elife*. 2017 **6**:e26111. [PMID: 28527238]
- [8] Kumar R *et al. Malar J*. 2002 **1**:5. [PMID: 12057017]
- [9] Munoz C *et al. Int J Parasitol*. 2012 **42**:715. [PMID: 22713760]
- [10] Delorme V *et al. Microbes Infect*. 2002 **4**:271. [PMID: 11909736]
- [11] Blisnick T *et al. Cell Microbiol*. 2006 **8**:591. [PMID: 16548885]
- [12] Das A *et al. J Cell Sci*. 1994 **107**:3477 [PMID: 7706399]
- [13] Mitsuhashi S *et al. J Biol Chem*. 2003 **278**:82 [PMID: 12374792]
- [14] Das I *et al. Science* 2015 **348**:239. [PMID: 25859045]
- [15] Vagnarelli P *et al. Cell* 2018 **174**:1049. [PMID: 30142342]
- [16] Qureshi R *et al. Biochem Biophys Res Commun*. 2019 **516**:770. [PMID: 31253400]
- [17] Tasdemir D *et al. Antimicrob Agents Chemother*. 2006 **50**:1352 [PMID: 16569852]
- [18] Webb B and Sali A, *Methods Mol Biol*. 2017 **1654**:39. [PMID: 28986782]
- [19] Fiser A and Sali A, *Bioinformatics*. 2003 **19**:2500. [PMID: 14668246]
- [20] De Wever V *et al. Biochem Biophys Res Commun*. 2014 **453**:432. [PMID: 25281536]
- [21] Stefan M *et al. Journal of Biological Chemistry*. 2001 **276**:3017. [PMID: 11069926]
- [22] Meiselbach H *et al. Chemistry & Biology*. 2006 **13**:49 [PMID: 16426971]
- [23] Hendrickx A *et al. Chemistry & Biology*. 2009 **16**:365. [PMID: 19389623]



Edited by P Kanguane

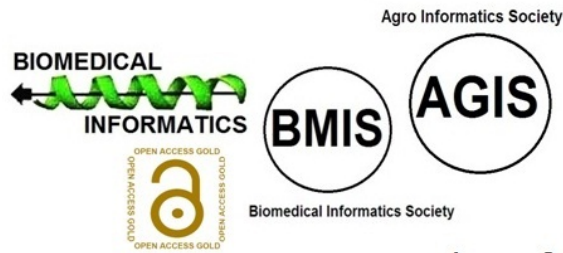
Citation: Qureshi *et al.* Bioinformation 16(11): 942-948 (2020)

License statement: This is an Open Access article which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited. This is distributed under the terms of the Creative Commons Attribution License

Articles published in BIOINFORMATION are open for relevant post publication comments and criticisms, which will be published immediately linking to the original article for FREE of cost without open access charges. Comments should be concise, coherent and critical in less than 1000 words.

BIOINFORMATION

Discovery at the interface of physical and biological sciences



since 2005

BIOINFORMATION

Discovery at the interface of physical and biological sciences

indexed in

