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Inferring Bisphenol-A influences on estrogen-mediated signalling in estrogen and androgen receptors: an *in silico* approach

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1. Introduction

Bisphenol A (BPA) is used in the polycarbonate (PC) plastics as monomer and in epoxy resins as additive. Due to the wide applications of PC plastics and epoxy resins in food contact plastics, the rate of BPA exposure to humans is high. The use of BPA in food contact plastics is restricted in 40 countries especially those used for children (Mahamuni and Shrinithivihahshini, 2017). BPA is a well-known endocrine disrupting compound (EDC) which causes high degree of health risk. BPA is structurally similar to 17β-estradiol (estrogen hormone or EST) with phenolic moiety. Because of this structural similarity, BPA interacts with estrogen receptor (ER) as agonist and alters down-stream expressions (Li et al., 2012, 2013; Ferris et al., 2015). ER present in various types of tissues and their subtypes may present in differential cell types of the same tissue. The estrogen receptor has two subtypes *viz.*, ERα and ERβ. The two subtypes of ER share 97% similarity in amino acid composition of the DNA binding domain (DBD), 56% similarity in the ligand binding domain (LBD) and 26% at the N-terminus region. Hence, the binding and downstream functional properties of ERα and ERβ proteins are likely to affected by misrecognition of any ligands similar to estrogen hormone. The ERα present mainly in uterus, stroma of prostate, theca cells of ovary, Leydig cells of testis, epididymis, bone, breast, brain, liver, adipose tissues etc., whereas ERβ mainly present in colon, epithelial tissues of prostate, testis, granulosa of ovary, bone marrow, salivary gland, vascular endothelium, brain etc. (Dahlman-Wright et al., 2006).

The androgen receptor (AR) is also a nuclear receptor responds to male reproductive hormones, testosterone (T) or dihydrotestosterone (DHT). Further, the binding of estrogen hormone to androgen receptor plays a significant role in maintaining various developmental male sexual characteristics. In addition to the similarity with estrogen, BPA also shares a partial similarity with testosterone and dihydrotestosterone with hydroxyl group as functional group. BPA is also found to have antiandrogenic property and significantly reduces the AR functions (Teng et al., 2013).

The nuclear steroid receptors, estrogen receptor and androgen receptor are playing significant role during adolescent and gestation stages. Hence, the interference with these receptors by BPA may bring serious steroid signalling metabolic disorders in the developmental stages of both sex. Estrogen receptor has been associated with various types of cancer, osteoporosis, neurodegenerative diseases (stroke, Parkinson disease, Alzheimer disease), cardiovascular diseases, obesity, male and female reproductive disorders (Deroo and Korach, 2006). AR has associated with prostate cancer, muscular atrophy, hypogonadism etc. (Shukla et al., 2016). Significantly, risks associated with male infertility due to BPA exposure necessitate further inquiries (Cariati et al., 2019). Though number of evidences has been produced on the endocrine disruption activity related with nuclear receptors ER and AR, the underlying molecular mechanism of interactions are less understood. There were several studies attempted to explore the BPA interactions with nuclear receptors using appropriate *in silico* techniques (Babu et al., 2012; D'Cruz et al., 2012; Vutukuru et al., 2016). Hence, in this article we made an attempt by adopting molecular docking techniques to further explore the molecular interactions of BPA with ER and AR proteins for possible interference of estrogen-mediated steroid signalling pathway.

2. Materials and methods

2.1. Protein-protein interaction network construction

The protein interaction network of Bisphenol A targeted proteins/genes was initially extracted from STITCH 4.0 database; where proteins were considered for only human related interactions. The STITCH data was extracted at high degree of confidence level excluded text mining. The other parameters were set as default. Eight proteins were selected from STITCH database in such a way that having a high confidence scores of 0.8–1.0 level. Further, networking individual protein interactions of each protein was achieved by STRING database. Network was extracted at a high degree of confidence level (0.7) excluded text mining. Each 8 networks were imported to Cytoscape (*V 3.2.3*; an open source software platform enables visualization of complex networks). All the networks were merged together into a total protein interaction network and proteins those were not in the total network were deleted. The resulted network was used for further analysis.

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2.2. Network analysis

2.2.1. Subnetwork/cluster analysis

The clusters of the BPA targeted protein network were identified using the plug-in MCODE. The cut off degree was set as two and cut off node score was set as 0.2 with high depth from the seed (100). The Haircut parameter was set true and the K-cut value was set at 2.0.

2.2.2. Functional enrichment analyses

The functional enrichment analyses were carried out using *ClueGO 2.1.7* plug-in in the Cytoscape platform. These are tools for identifying and visualizing enriched GO terms in ranked lists of genes. The enrichment applied in ClueGo test was Two-sided hypergeometric test with Bonferroni step down correction. The minimum GO level was set at 7 and maximum of 15. The kappa score threshold was set at 0.4. The GO used in ClueGO study was BiologicalProcess_06.10.2015_14h29: 17234.

2.3. Receptor protein screening

The human steroid receptors ERα, ERβ and AR were selected for the study. From all the available protein entries in the Research Collaboratory for Structural Bioinformatics: Protein Data Bank (RCSB-PDB) database, selective PDB entries involved in steroid hormone mediated signalling pathway were selected. After screening, 60 entries for ERα, 24 entries for ERβ and 75 entries for AR were selected for the docking study.

2.4. Protein preparation and grid generation

All the docking analyses were performed using Glide tool of Schrodinger-Maestro (*ver 10.7*) package. Maestro is one of the versatile molecular modelling environments with detailed intermolecular details offered. The protein structure was pre-processed with Protein preparation wizard tool of Maestro. Previously, the crystal structures of the proteins were imported from RSCB-PDB website. During pre-process, hydrogens were added, bond orders were assigned and water molecules beyond 5 Å from the hetero groups were deleted. Pre-processed crystal structure was optimized and the restrained minimisation was achieved using OPLS 2005 force field. Since, most PDB entries were found with ligands with agonistic binding sites, grid was generated in centroid of the same binding site. For those entries lacking ligands, sitemap scores were calculated using Site map tool of Maestro and best scored site was chosen for grid generation.

2.5. Ligand preparation

Bisphenol A structure was sketched using Maestro 2D sketcher, aligned and screened. Then the structure was imported and processed with default parameters of Ligprep tool. OPLS 2005 force field was applied during ligand preparation. The possible ionisation states of BPA and estradiol were derived at pH 7.0 (± 0.2) using Epik tool and used for further docking studies.

2.6. Protein-ligand docking

Prepared protein and BPA structures along with generated grids were used as input for Glide-Ligand docking tool of Maestro. The OPLS 2005 force field was used in this system and an extra precision method (XP) was chosen as scoring function. An extra precision XP Glide scoring was applied while docking, and the detailed scoring functions were described earlier (Friesner et al., 2006).

$$\text{XP Glide Score} = E_{coul} + E_{vdW} + E_{Bind} + E_{penalty}$$

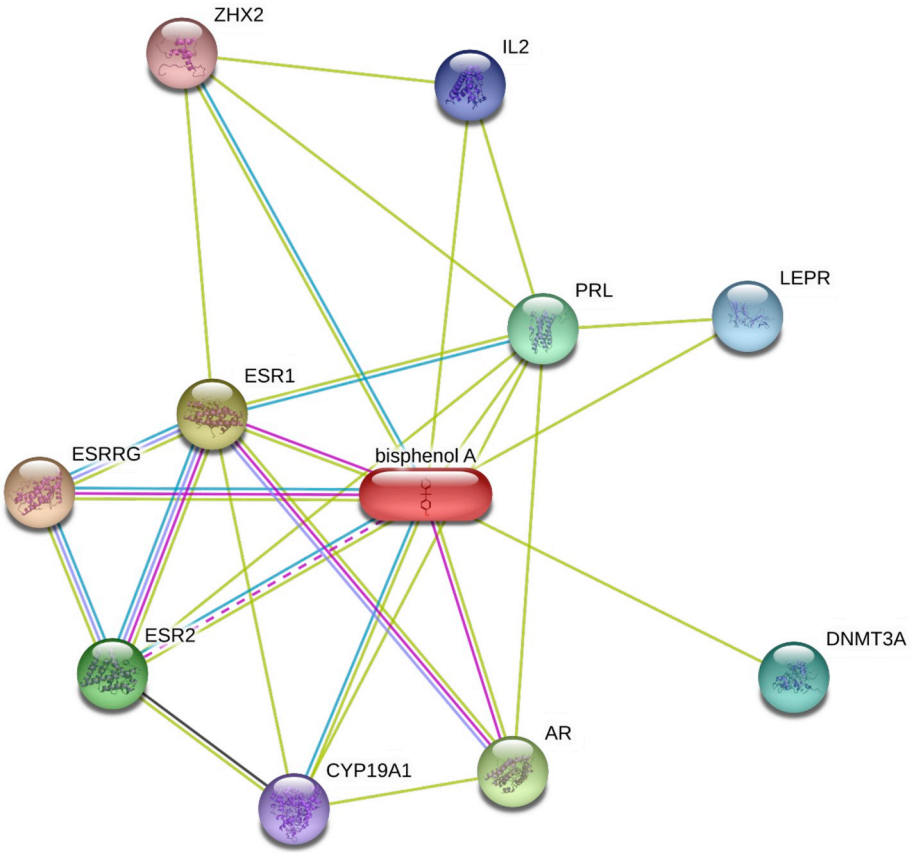

Fig. 1. Network of BPA-interacting human proteins resulted by STITCH database.

Table 1
STITCH scores of BPA interacting proteins with high confidence.

Top scored BPA-interacting proteins	STITCH score	Number of protein interactions found in STRING database
ESRRG	0.984	603
ESR1	0.938	237
AR	0.886	193
ESR2	0.864	554
PRL	0.814	285
DNMT3A	0.814	64
LEPR	0.8	279
IL2	0.8	420

where

$$E_{Bind} = E_{hyd\_enclosure} + E_{hb\_nn\_motif} + E_{hb\_cc\_motif} + E_{PI} + E_{hb\_pair} + E_{Phobic\_pair}$$

and

$$E_{penalty} = E_{desolv} + E_{ligand\_strain}$$

The docking job was submitted to a cluster node of High-Performance Computing Facility (HPC) and results were acquired. The ligand docking tool generate coordinates and confirmations for all the ligand poses at the specified binding site within the grid potentials. The bound complexes of all ligand poses were ranked through hierarchical filters and scored. The protein-ligand complex structure was analysed using Ligand Interaction Diagram and numeric results were derived using Glide-XP Visualiser tools of Maestro. The results were compiled and those entries with high XP Glide Scores were docked with estradiol for comparison. The protein-ligand complexes were exported as *.pdb* format and the additional structural views were achieved using UCSF Chimera (*ver. 1.8*) molecular visualisation application. Adobe Photoshop *CS6* software was used for image processing and compilation.

3. Results

3.1. BPA-targeted human proteins network

Initially, BPA interacting protein targets were identified using STITCH database, where human (*Homo sapiens*) proteins were selected with high confidence score, excluded text mining and not more than 10 interactions (Fig. 1). The proteins with high confidence STITCH score ranged between 0.7 and 1.0 were selected. Eight proteins were resulted in scores 0.8–1.0 and their corresponding STITCH scores were given in Table 1. The STITCH network of BPA interacting proteins contains 10 nodes, 21 edges with an average node degree of 4.2. The actual number of edges are more than the expected number of edges, thus the network has more significant interactions with a PPI enrichment P-value of 0.00462. Further, extending protein-protein networks of these top scored BPA interacting proteins were derived from STRING database.

The analysis of BPA interacting proteins derived from STITCH database explored the Gene Ontology (GO) terms related with these proteins. The GO terms were analysed by categories namely Biological processes, cellular processes, Molecular processes, KEGG pathways, Protein domains and Features and PFAM protein domains. Among 35 biological process GO terms, Steroid hormone mediated signalling pathway was highly significant (P-value of 4.66E-18). The Cellular

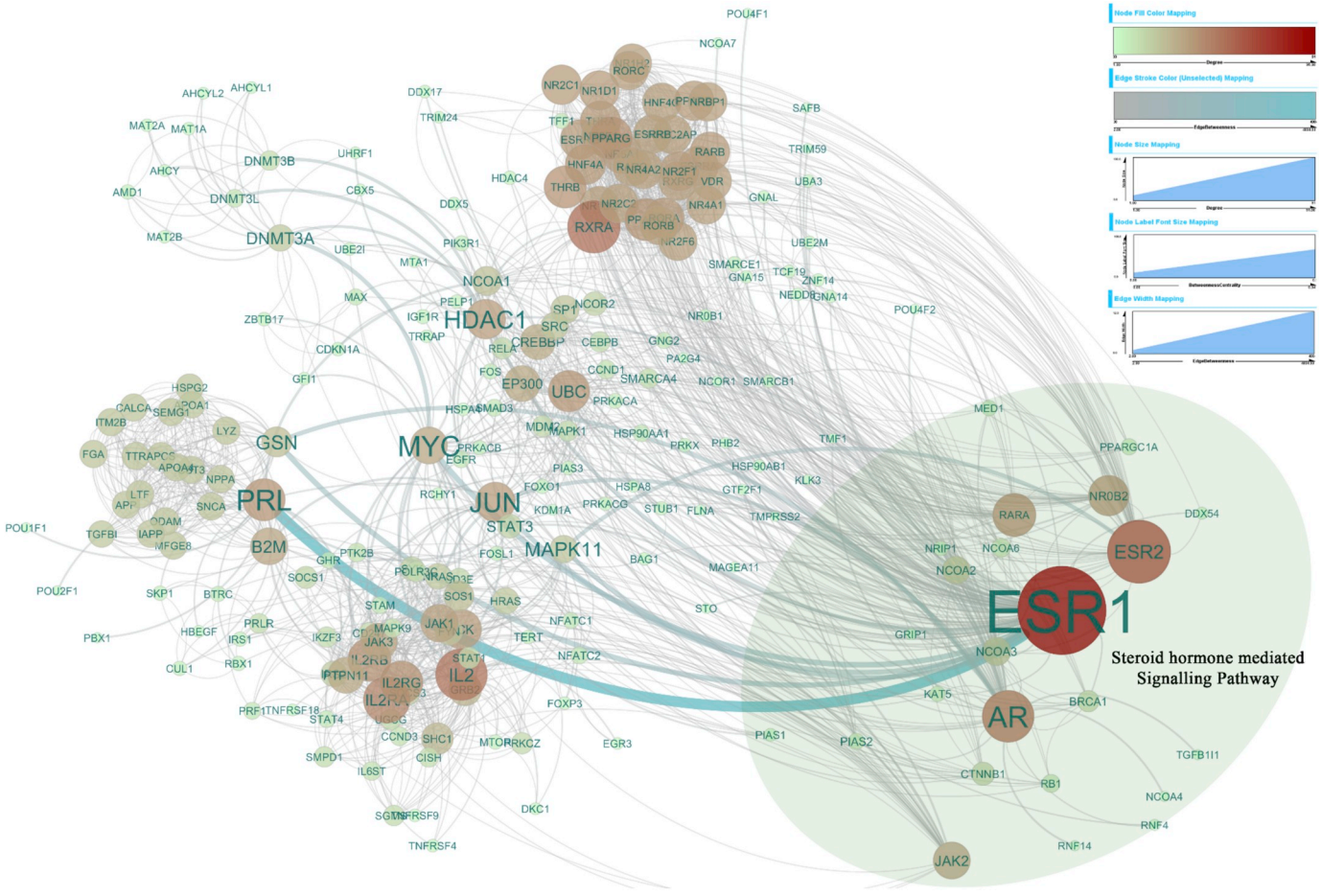

Fig. 2. Total Protein-Protein Interaction Network of top-scored BPA interacting proteins merged in CYTOSCAPE software.

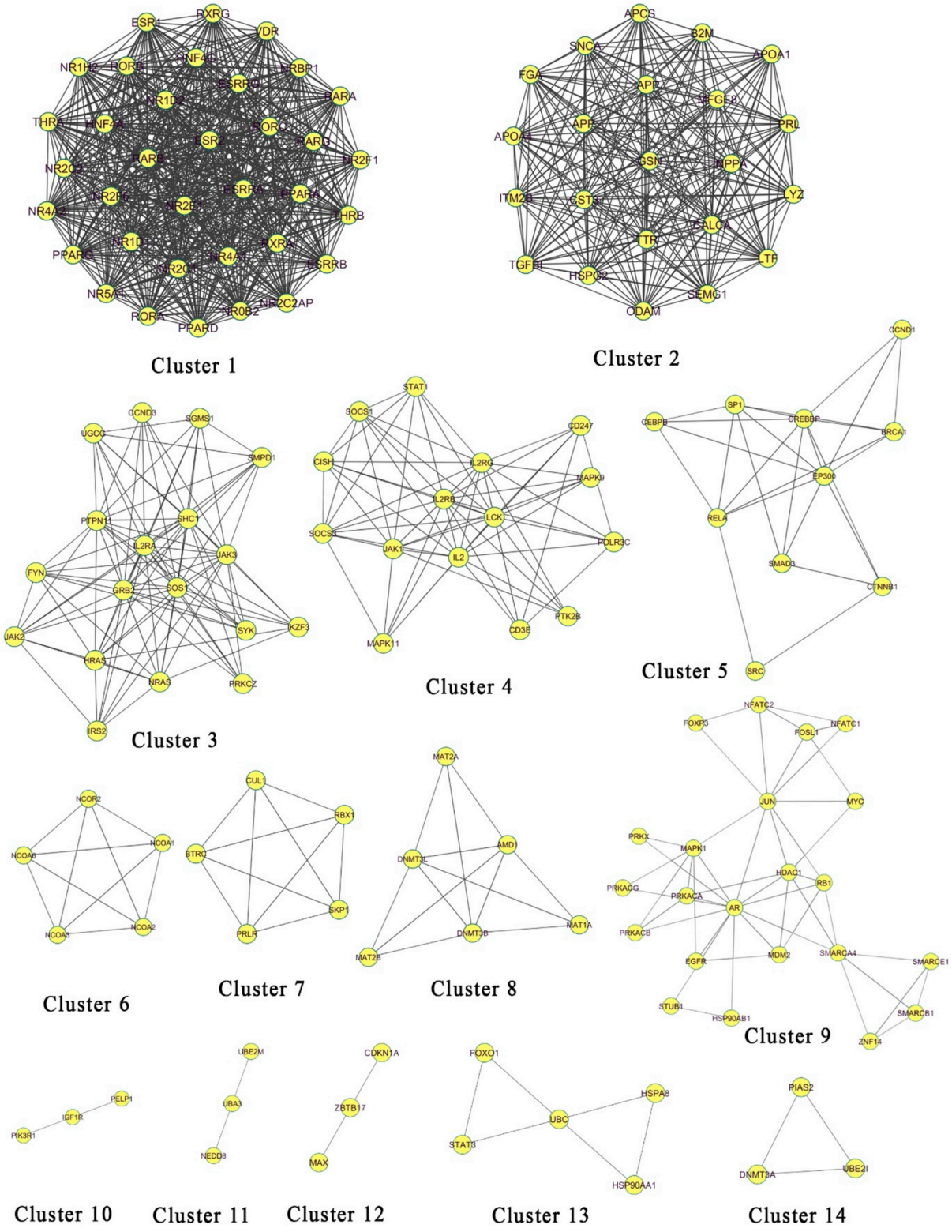

Fig. 3. Clusters/Subnetworks extracted from total PPI network of BPA interacting proteins using MCODE plug-in.

process GO terms signify the term 'nucleoplasm' with a P-value of 0.000775. Similarly, the term 'steroid hormone receptor activity' was significantly related with a P-value of 9.89E-19 among 26 Molecular processes related terms. The protein domains and features analysis showing that 'Nuclear hormone receptor, ligand-binding domain' term was highly related (P-value 2.09E-17) with these BPA interacting proteins. Pfam protein domain analysis was also found that 'Ligand-binding domain of nuclear hormone receptor' term was highly related

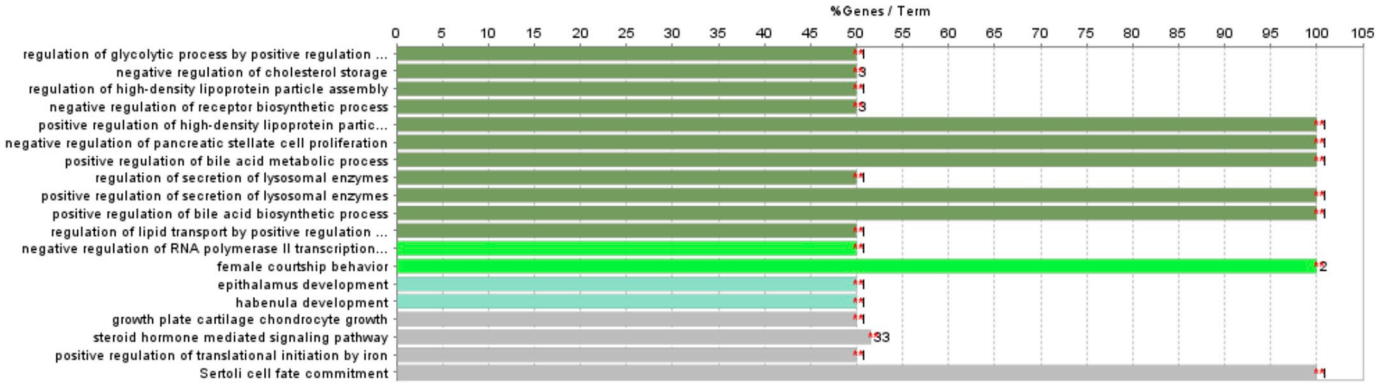

Fig. 4. Gene Ontology terms related to Cluster1 of MCODE functionally enriched in ClueGO Plug-in.

(P-value 9.79E-18) to these proteins. Collectively all these findings reveal that BPA targets the proteins, which are mainly involved in the 'steroid hormone mediated signalling'.

3.2. Total PPI network of BPA-interacting proteins

The PPI networks of eight BPA interacting proteins were imported into Cytoscape Software and merged together to get a total PPI network. the merged network was containing 255 nodes and 2069 edges. The proteins (32 nodes with 278 edges) outside the total network were deleted and the final network was used for further analysis. For better visualization, a plug-in called EnViz (Enriched Visualization) was used (Fig. 2). During visualization, the node size and node colour was mapped with Degree of networking. The final PPI network contains 223 nodes and 1791 edges. High degree of networking was contented by ESR1 (91), ESR2 (62), RXRA (50), AR (49) and IL2 (49) proteins as top 5 members of the total PPI network. The degree is the indirect measure of number of edges connected with the particular node.

3.3. Sub-network/Cluster analysis

The total PPI network was subjected to cluster analysis using MCODE plug-in. The MCODE tool identified 14 distinct clusters from the total protein network of BPA interactions (Fig. 3). The top ranked cluster has 35 nodes and 595 edges and has a highest MCODE score of 35. The clusters were further analysed for functional enrichment.

3.4. Functional enrichment analysis of total PPI network

ClueGo is a Cytoscape plug-in helps to interpret the biological functions of a set of genes. It is an integrated platform of Gene Ontology (GO) and KEGG/BioCarta pathways resulted in well-organized functional enrichment (Bindea et al., 2009). The gene ontology used in this study was BiologicalProcess_06.10.2015_14h29. There were 17234 unique genes in this GO. The enrichment method used in this study is two-sided hypergeometric enrichment/deletion with Bonferroni step down correction method. The Cluster 1, extracted from MCODE was containing 35 genes, all of which found to present in this GO. The number of genes present in all the clusters after selection was found as 33 (94.29%). There were 19 GO terms specific to Cluster 1, in addition with 11 related terms were also found (Fig. 4). At the maximum 33 gene were found to associated with steroid hormone mediated signalling pathway by this high ranked subnetwork. The respective Term P-Value for this steroid hormone mediated signalling pathway is 1.49E-82, which is highly significant among the all 19 terms (Table 2). Receptor proteins involved in this steroid hormone mediated signalling pathways are further screened and their molecular interactions with BPA were studied through docking experiments. The 'negative regulation of cholesterol storage', 'negative regulation of

Table 2
GO terms related to Cluster 1 with significance details enriched by ClueGO plug-in.

GOTerm	GOLevels	Nr. Genes	% Associated Genes	Term PValue
growth plate cartilage chondrocyte growth	(4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14)	1	50	0.004057732
female courtship behavior	(5, 6, 7)	2	100	4.01E-06
negative regulation of receptor biosynthetic process	(4, 5, 6, 7, 8)	3	50	1.53E-07
negative regulation of cholesterol storage	(3, 4, 5, 6, 7)	3	50	1.53E-07
negative regulation of RNA polymerase II transcriptional preinitiation complex assembly	(7, 8, 9, 10, 11, 12, 13, 14)	1	50	0.004057732
epithalamus development	(3, 4, 5, 6, 7, 8, 9, 10)	1	50	0.004057732
habenula development	(3, 4, 5, 6, 7, 8, 9, 10, 11)	1	50	0.004057732
steroid hormone mediated signalling pathway	**(5, 6, 7)**	**33**	**51.5625**	**1.49E-82**
positive regulation of translational initiation by iron	(5, 6, 7, 8, 9, 10, 11)	1	50	0.004057732
Sertoli cell fate commitment	(3, 4, 6, 7, 8, 9, 10)	1	100	0.002030869
positive regulation of bile acid biosynthetic process	(5, 6, 7, 8, 9, 10, 11)	1	100	0.002030869
regulation of glycolytic process by positive regulation of transcription from RNA polymerase II promoter	(6, 7, 8, 9, 10, 11, 12, 13, 14, 15)	1	50	0.004057732
regulation of lipid transport by positive regulation of transcription from RNA polymerase II promoter	(5, 6, 7, 8, 9, 10, 11, 12, 13)	1	50	0.004057732
regulation of high-density lipoprotein particle assembly	(3, 4, 5, 6, 8)	1	50	0.004057732
positive regulation of high-density lipoprotein particle assembly	(3, 4, 5, 6, 7, 8, 9)	1	100	0.002030869
regulation of secretion of lysosomal enzymes	(6, 7, 8, 9)	1	50	0.004057732
positive regulation of secretion of lysosomal enzymes	(5, 6, 7, 8, 9, 10)	1	100	0.002030869
positive regulation of bile acid metabolic process	(4, 5, 6, 7, 8, 9, 10)	1	100	0.002030869
negative regulation of pancreatic stellate cell proliferation	(5, 6, 7)	1	100	0.002030869

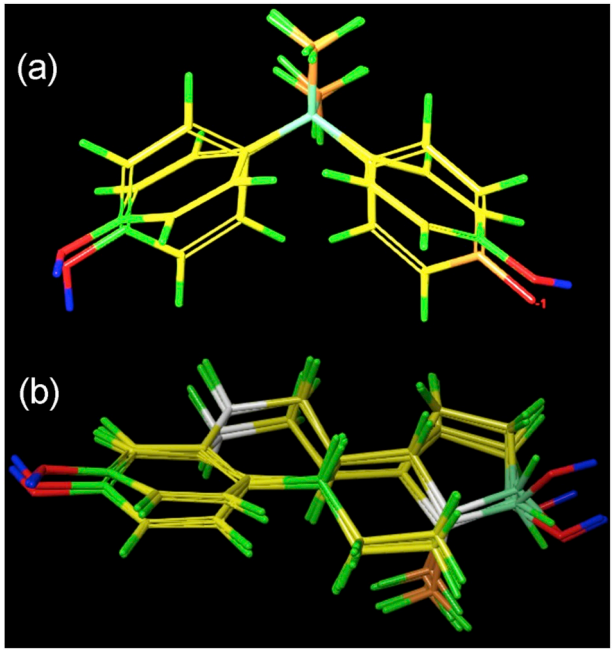

Fig. 5. Conformational variations of (a) Bisphenol A and (b) 17β-estradiol resulted in Epik ionization states during ligand preparation.

receptor biosynthetic process' and 'female courtship behaviour' are some of the other highly significant GO terms followed by 'steroid hormone mediated signalling pathway'.

3.5. Protein screening for steroid hormone mediated signalling

The functional enrichment analyses of BPA-targeted proteins from STITCH revealed the potential influence of BPA in the 'steroid hormone mediated signalling'. Similarly, the functional enrichment of total PPI network of BPA-targeted proteins by ClueGO was also revealed the similar potential influence on steroid hormone mediated signalling pathway. Next to this, 'negative regulation of receptor biosynthetic process' and 'negative regulation of cholesterol storage' are the significant GO terms predicted by ClueGO, to be involved by the subnetwork of BPA interacting proteins. Already, few synthetic compounds including Bisphenol A have been suspected as obesogens and are related to fat accumulation and obesity (Stanek et al., 2015). The highly significant relationship of BPA interacting proteins and the biological implications of cholesterol metabolism confirms the involvement of BPA in the interferences of fat accumulation and obesity. In order to explore further the molecular interactions, nuclear receptors involved in 'steroid hormone mediated signalling pathway' were screened from all the available RCSB-PDB web database. It was found that among eight receptor proteins, only ERα, ERβ and AR were involved in this pathway.

The search algorithm and scoring functions are the key factors in the protein-ligand docking (Du et al., 2016). The search algorithm in the Glide tool is used to find suitable binding site with the ligand confirmation was selected through hierarchical filters. An extra precision Glide scoring was applied in all the docking experiments. During ligand preparation, two different conformational poses for BPA and four different conformational poses for 17β-estradiol were identified (Fig. 5). Of these, one of the BPA confirmation is found to be negatively charged at the first oxygen (O1) by losing its hydrogen atom. The scoring system is used to determine the binding affinity of the generated conformational poses of the ligands.

Table 3
Docking Scores for ERα, ERβ and AR receptors with possible conformational variations of BPA and estradiol (EST).

Receptor	Protein Ligand Complex	XP Glide Score	Glide Energy (Kcal/mol)
ERα	*2yja*_BPA1	−09.85	−35.70
	*2yja*_BPA2	−08.76	−28.68
	*2yja*_EST1	−11.53	−51.55
	*2yja*_EST2	−11.37	−50.32
	*2yja*_EST3	−10.75	−45.93
	*2yja*_EST4	−10.54	−43.05
ERβ	*1u9e*_BPA1	−10.00	−40.28
	*1u9e*_BPA2	−09.80	−36.60
	*1u9e*_EST1	−11.31	−41.90
	*1u9e*_EST2	−11.04	−43.82
	*1u9e*_EST3	−10.51	−40.95
	*1u9e*_EST4	−10.36	−39.32
AR	*2ylq*_BPA1	−11.72	−33.59
	*2ylq*_BPA2	−09.79	−40.26
	*2ylq*_EST1	−11.59	−47.27
	*2ylq*_EST2	−11.07	−44.59
	*2ylq*_EST3	−10.89	−44.63
	*2ylq*_EST4	−10.72	−42.06

The docking score is simply the XP Glide score without inclusion of Epik state penalties. There were 59 PDB entries for ERα docked against BPA, where the binding affinity values ranged from −05.4 to −09.86 in terms of XP Glide score. Among 59 entries of ERα docked, *2yja* yielded high XP Glide score (−09.86). Similarly, 24 ERβ entries for ERβ were docked against BPA, where XP Glide score ranged from −03.54 to −10.00. In ERβ, *1u9e* scored higher XP Glide values. Likewise, 71 AR entries representing AR protein were docked against BPA, where the XP Glide score ranged from −07.79 to −11.72 and *2ylq* resulted with high scores comparatively (Table 3). The top scored entries were then docked with natural ligand estradiol (estrogen) for comparison. The XP glide scores for estrogen receptor exhibit higher binding affinity towards estradiol than BPA in estrogen α and β subtypes. The binding energy of estradiol is also higher than that of BPA. Though the Glide scores and bound energy of the ER-EST complexes are higher than the ER-BPA complexes, they are relatively analogues to BPA complexes. In contrast, the androgen receptor exhibits slightly higher affinity towards BPA than estradiol. Though the Glide score of androgen receptor with BPA is relatively higher than estradiol, the Glide energy is lesser.

The study revealed that both the ligands estradiol and BPA binds to the active sites of the receptors (ERα, ERβ and AR) which are always encompassed with hydrophobic amino acid residues (Figs. 6 and 7). The residues of the binding site in Figs. 6 and 7 were coloured as per kd-Hydrophobicity (Kyte-Doolittle Hydrophobicity) using UCSF Chimera software. In the docked complexes, BPA and estradiol showed some conformational changes to fit into the binding sites than from the confirmation of prepared ligand structures (Table 4). The distance between oxygen atoms of hydroxyl group of bisphenol A was slightly increased to relatively match the estradiol confirmation (Table 5).

3.6. Protein-ligand interactions in ERα complex

The ligand interaction diagrams of ERα with BPA and with estradiol were depicted in Fig. 8-a and 8-b respectively. The phenolic ring A of BPA interacts with *Gly521* and *Glu353* of the backbone through hydrogen bonds (Table 6). It also creates *Pi-Pi* interaction with phenolic ring of *Phe404*. The negatively charged oxygen atom in *Glu353* serves as acceptor whereas the positively charged H_2N^+ group of *Gly521* serves as donor for hydroxyl group of the A-ring of BPA. The B-ring of BPA

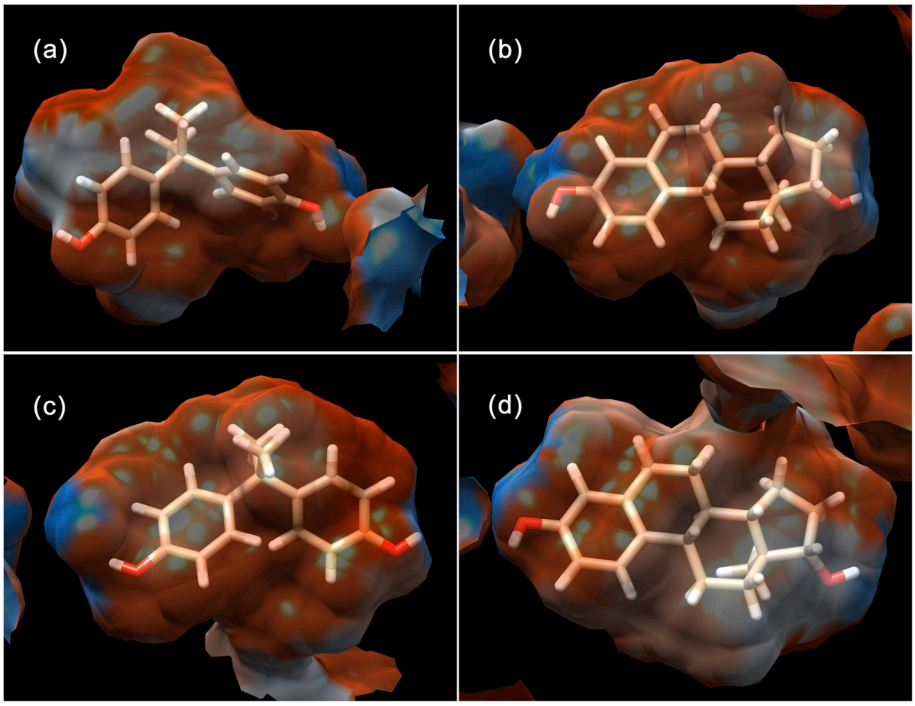

Fig. 6. The protein-ligand complex of (a) ERα-BPA, (b) ERα-EST, (c) ERβ-BPA and (d) ERβ-EST, in their respective binding site enclosed by hydrophobic amino acids (coloured as per kdHydrophobicity).

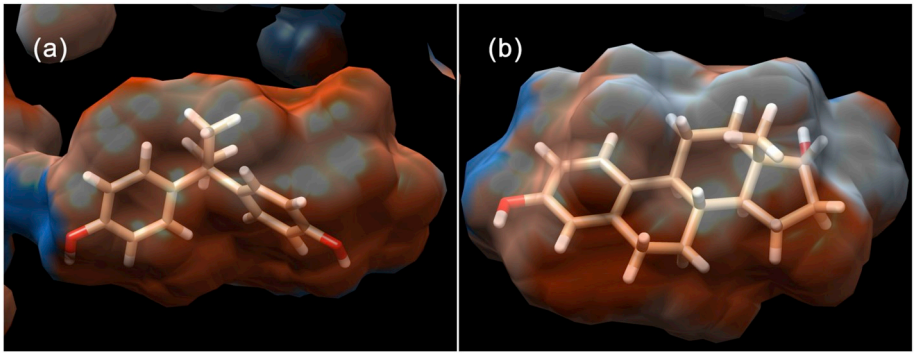

Fig. 7. The protein-ligand complex of (a) AR-BPA and (b) AR-EST in their respective binding site enclosed by hydrophobic amino acids (coloured as per kdHydrophobicity).

Table 4
Distance between the hydroxyl groups (measured between the oxygen atoms) present in both the ligands prior to docking.

Prepared Ligand confirmations	Distance between hydroxyl groups, Å
BPA1	09.29
BPA2	09.29
EST1	11.04
EST2	10.96
EST3	10.45
EST4	10.84

interacts with *Arg394* of the side chain through a hydrogen bond where the oxygen of the amino acid serves as donor. A similar interaction is shown by the estradiol, where A-ring interacts through hydrogen bonds with *Gly521* and *Glu353* and a *Pi-Pi* interaction with *Phe404*. Instead of *Gly521* interacting with BPA, *His524* is creating a hydrogen bond with D ring of estradiol. Here, the hydroxyl group of estradiol serves as donor.

Table 5
Distance between the oxygen atoms of two hydroxyl groups of both ligands shows conformational changes after docking.

High scored dock complexes	Distance between hydroxyl groups, Å	Difference, Å
*2yja*_BPA1	09.66	0.37
*2yja*_EST1	11.10	0.06
*1u9e*_BPA1	09.65	0.36
*1u9e*_EST2	10.98	0.02
*2ylq*_BPA2	10.01	0.72
*2ylq*_EST2	10.38	−0.58

3.7. Protein-ligand interactions in ERβ complex

The Fig. 8-c and 8-d are depicting the ligand interaction diagram of ERβ with BPA and estradiol respectively. The ring A of BPA interacts with *Glu305* of the side chain through a hydrogen bond and *Phe356* creates a *Pi-Pi* interaction (Table 6). Ring B of BPA creates a hydrogen bond with *Hie475* of the backbone. The negatively charged oxygen

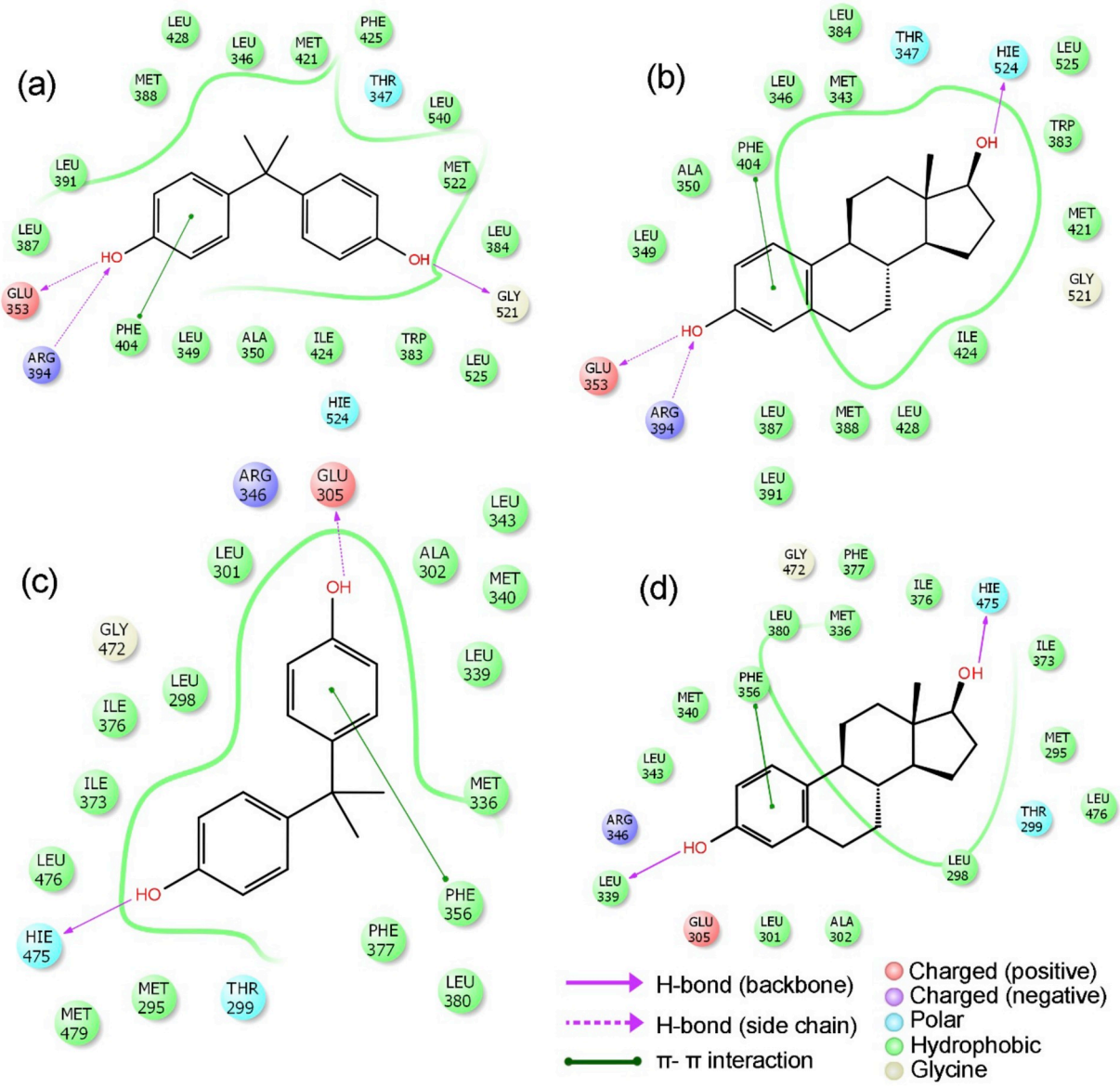

Fig. 8. Ligand interaction diagram of ERα, and ERβ protein residues from Glide docking.

atom of *Glu305* and nitrogen of *Hie475* serves as acceptors in creating the hydrogen bonds. As in ERα, here also similar protein-ligand interactions with BPA and estradiol took place except *Leu339* is bonded with hydroxyl group of D-ring of estradiol whereas *Glu305* is bonded with A-ring of BPA.

3.8. Protein-ligand interactions in AR complex

The Fig. 9 (a and b) is depicting the ligand interaction diagram of AR with BPA and estradiol. The A-ring of bisphenol A create hydrogen bonds with *Gln711* and *Arg752* and the B-ring creates hydrogen bond with *Asn705* of the side chain (Table 6). The estradiol also interacts in a similar manner to that of BPA with AR. The A-ring of estradiol creates a hydrogen bond with *Met745* of the backbone. Unlike BPA, A-ring of estradiol create a *Pi-Pi* interaction with *Phe764*.

4. Discussion

The network of BPA-targeted human proteins was identified using STITCH and STRING databases and their protein-protein interaction (PPI) network was constructed using CYTOSCAPE software. The network was found to have significant number of nodes and edges with good integrity. From STITCH database analysis, it was found that among 35 GO terms related with the BPA-targeted proteins, 'steroid signalling pathway' was found with high significance. The analysis of total PPI network analysis also suggests that proteins involved in the network are highly interactive among in nature. Some of the animal studies conducted earlier were supporting that BPA can significantly affects the concentration of steroid hormones, expression of steroid hormone receptors and may results in reproductive abnormalities (Salian-Mehta et al., 2014; Miki et al., 2016; Patel et al., 2017).

The total PPI network has few clusters and highly ranked sub clusters were subjected to functional enrichment analysis. The results suggest that a major number of genes in the cluster are found to be associated with steroid hormone mediated signalling pathway. The 'negative regulation of cholesterol storage', 'negative regulation of receptor biosynthetic process' and 'female courtship behaviour' are some of the other highly significant GO terms next to 'steroid hormone mediated signalling pathway'. The ovarian and testicular steroid homeostasis was found to be affected by BPA as reported earlier (Salian-Mehta et al., 2014). Especially the overall reproductive health is at risk as xenoestrogens lie BPA alters the steroid signalling pathways

Table 6
Bond length comparison of protein-ligand complexes of estrogen and androgen receptors with tested ligands.

Receptor	Protein Ligand Complex	H-bond Interaction residues	H-bond (D—H···A)	H-bond length, Å	*Pi-Pi* Interaction residues
ERα	*2yja_BPA*	*Glu353*[a]	*OH···O⁻(Glu353)*	*1.97*	Phe404
		Arg394[a]	*H_2N(Arg394)···OH*	*2.36*	
		Gly521	*OH···O(Gly521)*	*1.76*	
	2yja_EST	*Glu353*[a]	*OH···O⁻(Glu353)*	*1.79*	Phe404
		Arg394[a]	*H_2N(Arg394)···OH*	*1.93*	
		Hie524	*OH···N(Hie524)*	*1.94*	
ERβ	*1u9e_BPA*	*Glu305*[a]	*OH···O⁻(Glu305)*	*2.66*	Phe356
		Hie475	*OH···N(Hie475)*	*1.88*	
	1u9e_EST	*Leu339*	*OH···O(Leu339)*	*2.06*	Phe356
		Hie475	*OH···N(Hie475)*	*2.25*	
AR	*2ylq_BPA*	*Asn705*[a]	*OH···O(Asn705)*	*1.71*	–
		Gln711[a]	*OH···O(Gln711)*	*2.64*	
		Arg752[a]	*H_2N^+(Arg752)···OH*	*2.34*	
	2ylq_EST	*Asn705*[a]	*OH···O(Asn705)*	*1.82*	Phe764
		Gln711[a]	*H_2N(Gln711)···OH*	*2.53*	
		Arg752[a]	*H_2N^+(Arg752)···OH*	*2.61*	
		Met745	*OH···O(Met745)*	*2.19*	

[a] -sidechain residues; otherwise-backbone residues; D– Donor; ···A-acceptor.

(Sidorkiewicz et al., 2018).

Earlier, the protein domains and features analysis from STITCH database analysis of BPA-targeted proteins found that 'Nuclear hormone receptor, ligand-binding domain' term was highly significant. Further, Pfam protein domain analysis was also found that 'Ligand-binding domain of nuclear hormone receptor' term was highly significant. Having these inferences, nuclear steroid receptors were screened for involvement in the steroid signalling pathway in order to study the further molecular interactions. After screening, we found that estrogen receptors (ER) – α, β and androgen receptors (AR) are found to involve in steroid signalling pathways. These receptors were subjected for molecular docking simulations for further understanding of molecular interactions.

The estrogen receptor subtypes share a common architecture of the nuclear receptor (NR) superfamily comprised of A to F regions (Ng et al., 2014). The well conserved regions E and F located at the C-terminus is consists both ligand binding domain (LBD) and ligand-dependent activation function2 (AF2) domains. The variations in these regions determines the specificity of the ligand interactions. The ligand binding cavity is mainly contributed by 12 helices (H1–H12) which offers high flexibility and enables ER subtypes respond to wide variety of ligands. This high flexibility explains how ER subtypes responds to BPA in a similar way to that of estradiol. In our study also, BPA was found to bind with the active binding site similar to Estradiol. In comparison, *Glu353* and *Arg394* for H-bond interactions and *Phe404* for *Pi-Pi* interactions are the common residues shared by both BPA and estradiol in ERα. Similarly, *Hie475* for hydrogen bond and *Phe356* for *Pi-Pi* interaction are the common residues shared by both BPA and estradiol in ERβ. Likewise, in AR, *Asn705*, *Gln711* and *Arg752* are the common amino acid residues in creating H-bonds shared by both BPA and estradiol. The phenolic moiety endowed by A-ring of BPA and estradiol can easily binds with the hydrophobically conserved binding site by forming hydrogen bonds. In our study, the docked complexes of ERα and ERβ clearly showed that the ligands bound within the binding site surrounded by the hydrophobic amino acid residues. The specificity of BPA with hydrophobic binding site of ER is experimentally demonstrated earlier. ERα with poorly constituted hydrophobic binding site docked with BPA resulted in antagonistic binding (Sengupta et al., 2013). In another study, Androstenedione (structurally similar to estrogen) does not bind to ER because of it has ketone instead of hydroxyl as functional group in the A-ring (Ng et al., 2014). Here, it explains that the phenolic moiety plays vital role in mimicking the estrogen hormone which is present in BPA. Like the estrogen receptor subtypes, AR is also having common architecture of the nuclear receptor superfamily (Lupien et al., 2007). It has a similar hydrophobically conserved region in the 12th helix in the ligand binding domain as like ER subtypes. BPA and estradiol are always bound within the hydrophobically conserved binding sites of AR protein.

The similarity between BPA and estradiol in presence of hydroxyl ions as functional groups enables BPA to competently interfere the estrogen-mediated ER function (Baker et al., 2012). It has been reported that BPA possess estrogenic properties and alters the hormonal functions of estrogen and androgen receptors. For example, the expressions of *ESR1* and *ESR2* (genes of ERα and ERβ respectively) are altered postnatally upon prenatal exposure of BPA in rats (Cao et al., 2013). This study found that mediobasal hypothalamus and amygdala of the developing brain of the rats are affected by the low-dose BPA exposure.

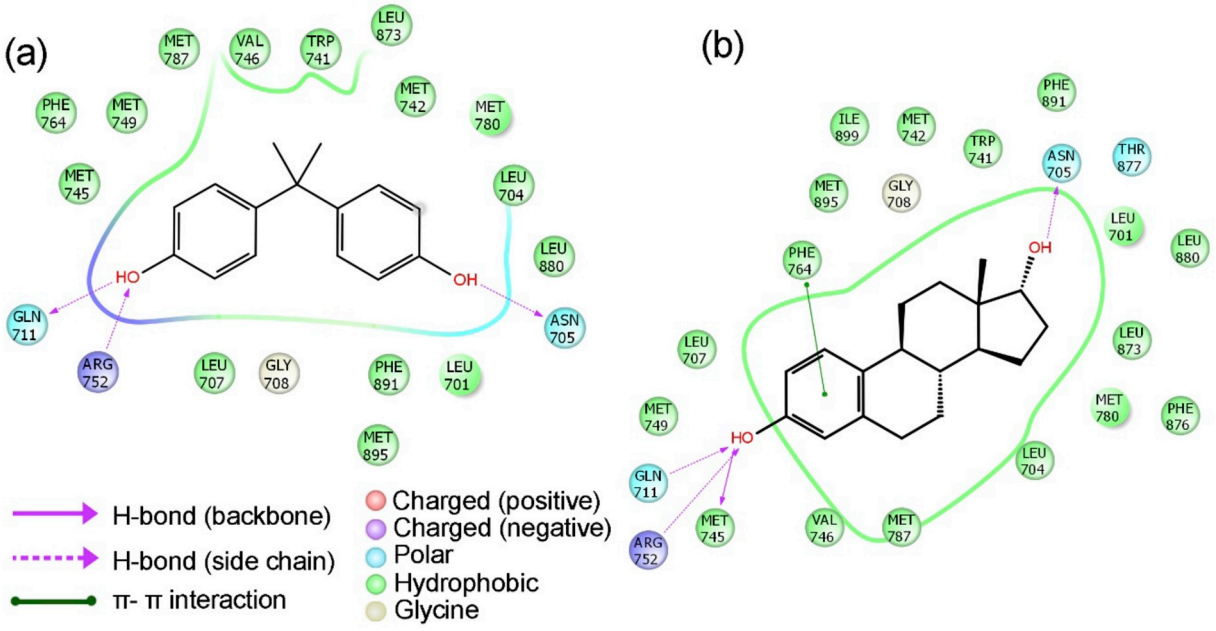

Fig. 9. Ligand interaction diagram of AR protein residue from Glide docking.

Likewise, the ERα-dependent NO-mediated synaptic responses were depressed by BPA which may result in the neuro-behavioural locomotor abnormalities (Pandey and Deshpande, 2015). Further, BPA influences the learning and memory functions, through increased DNA-methylation and decreased mRNA-expressions in ERα of the hippocampus (Chang et al., 2016). In addition to the impacts on brain and neuronal functions, BPA also influences the glycogen and lipid metabolism mediated by ER. For instance, BPA causes (i) rapid reduction in ERβ-mediated K_{ATP} channel activity, (ii) increased glucose-induced calcium-ion signals and (iii) enhanced insulin release in β-cells. These activities are reported higher in β-cells of islets of humans than mice even while same concentrations were used (Soriano et al., 2012). Likewise, liver metabolism is influenced upon BPA exposure by the upregulation of *CYP2C9* expression mediated by ERα expressions (Xu et al., 2016). ER-mediated sexual functions are also interfered by the BPA exposure. Bisphenol A exposure during spermatogenesis, increases the proliferation of sertoli cells (which involves in the cell growth and differentiation during spermatogenesis) by antagonising both ER-α and β (Ge et al., 2014). The immune responses were also found to be disturbed by BPA through ERα expressions (Yang et al., 2015). The carcinogenic nature of BPA also researched to some extent. BPA exposure in mice found to cause ovarian cancer by altering ERα expressions through interference of insulin-like growth factor-1 receptor (*IGF-1R*) signalling pathway (Hwang et al., 2013). Further, the ovarian cancer is caused by influences of BPA through both subtypes of ER expressions which induce the epithelial mesenchymal and migration of cancer cells (Kim et al., 2015). There were several reports that synthetic and natural molecules including BPA found to be agonist on both subtypes of ERs with several folds less affinity than the estrogen hormone (Teng et al., 2013; Hejmej et al., 2011) and the relative binding affinity is depending on the available free concentrations of any EDC (Kwon et al., 2007). The BPA is reported to have less potency in binding with ERα and its gene expressions than estradiol in an *in vivo* experiment (Gertz et al., 2012). Similar responses were obtained in our *in-silico* studies. The XP Glide scores obtained for ERα and ERβ with BPA in our study were lower than estradiol, which shows that both subtypes of ER receptors are having higher affinity towards estradiol than BPA (Table 1). Although one of the mutant types ERα subtype is having higher XP Glide score, only wild type proteins were considered for further analysis. In ERβ and AR proteins, wild type variants only scored higher docking score compared to mutant types. The details on wild and mutant type receptors used in this study and their respective scoring details given as appendices in supplementary information. Also, the bond length variations of *Glu353* and *Arg394* in ERα-BPA and ERα-EST complexes shows stronger affinity of receptor towards estradiol (Table 2). Though the affinity towards BPA is less, the ER-BPA complex is likely to produce similar downstream functional properties as like ER-EST complex, because the top ranked ER-BPA docking scores and the bound complex energy are relatively analogous to the former. ERβ is having slightly higher affinity than ERα towards BPA.

There are reports available on the involvement of estradiol in AR signalling as natural ligand that play vital role in the development male reproductive system (Yeh et al., 1998). Interestingly, AR is having slightly higher affinity towards BPA than ERα and ERβ in terms of XP Glide score. Evidently, the androgen receptor functions are influenced by BPA as antagonist just as estrogen receptor in laboratory experiments (Teng et al., 2013; Qiu et al., 2013; Kolšek et al., 2015). It is also found that the antagonistic expressions mediated by BPA were competitively reduced by the DHT (one of the natural ligands) in androgen receptor in a dose-dependent manner (Sun et al., 2006). This study indicates that BPA is having relative affinity towards androgen receptor even in the presence of male reproductive hormones. As a consequence, the number of mesenchymal androgen and estrogen receptors were increased with the increase of BPA exposure and also found interference of AR-estradiol interactions by BPA was observed in a dose-dependent manner (Richter et al., 2007). This implies that previously discussed similarity and relative affinity between estradiol and BPA towards estrogen receptor could also be exhibited in estrogen-mediated AR expressions. A stronger affinity of AR towards BPA than estradiol can be observed by examining the bond length variations of both complexes of AR with BPA and EST, where *Asn705* and *Arg752* of the AR side chains strongly bound by hydrogen bonds with BPA (Table 2). It can be considered that BPA could possibly outpaces the estradiol in AR expressions not necessarily the testosterone (T) or dihydrotestosterone (DHT), which are the natural male reproductive hormones. Further study on AR interaction with T and DHT compared with BPA may reveal the competency of BPA against these hormones.

5. Conclusion

The analysis of total protein-protein network of BPA-targeted human proteins suggests that these proteins are highly associated with 'steroid hormone-mediated signalling pathway'. The steroid receptors ERα, ERβ and AR were found to be involved in this pathway and subjected for molecular docking for a better understanding of molecular interactions with BPA and estrogen. The specificity of receptors to both the ligands is materialised by hydrophobic binding sites and the structural similarity in the phenolic moiety. The study revealed that BPA shares structural similarity and the specificity in binding in hydrophobic site with estradiol which makes BPA as a potential endocrine disrupting compound. The study also found that BPA interacts with steroid nuclear receptors ERα, ERβ and AR in similar way to estradiol. However, the higher affinity of AR towards BPA than estradiol lead us to the prediction that estrogen-mediated androgen receptor signalling and its downstream functional properties are likely to be affected highly by interferences of BPA in addition to ERα and ERβ. Further studies on BPA with male reproductive hormones are required to understand the BPA influences on AR activity.

Conflicts of interest

The authors are hereby declaring that do not have any conflict of interest.

Author contribution

DM – execution and interpretation of the research work and manuscript drafting; NDS- design of the research work, mentoring and manuscript preparation.

Acknowledgement

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Annexure 1. List of PDB residues of ERα, ERβ and AR proteins involved in steroid hormone-mediated signalling pathway with reference

Table 1
List of ESR1 (ERα) protein IDs involved in steroid hormone -mediated signalling pathway

Sl. No.	PDB ID	Reference/DOI
	1A52	10.1073/pnas.95.11.5998
	1ERE	10.1038/39645
	1ERR	10.1038/39645
	1G50	10.1006/prep.2001.1409
	1GWQ	10.1074/jbc.M200764200
	1GWR	10.1074/jbc.M200764200
	1PCG	10.1073/pnas.1934759100
	1QKT	10.1074/jbc.M009870200
	1QKU	10.1074/jbc.M009870200
	1R5K	10.1016/j.molcel.2005.04.014
	1SJ0	10.1021/jm034243°
	1UOM	10.1021/jm030086h
	1X7E	10.1021/ja047633°
	1 X 7R	10.1016/j.str.2004.09.015
	1XP1	10.1016/j.bmcl.2004.10.036
	1XP6	10.1016/j.bmcl.2004.10.036
	1XP9	10.1016/j.bmcl.2004.10.036
	1XPC	10.1016/j.bmcl.2004.10.036
	1XQC	10.1021/jm040858p
	1YIM	10.1016/j.bmcl.2005.01.046
	1YIN	10.1016/j.bmcl.2005.01.046
	2AYR	10.1021/jm050723z
	2BJ4	10.1073/pnas.0407189102
	2I0J	10.1021/jm060491j
	2IOG	10.1016/j.bmcl.2007.01.054
	2IOK	10.1016/j.bmcl.2007.01.054
	2JF9	10.1074/jbc.M611424200
	2JFA	10.1074/jbc.M611424200
	2OCF	10.1073/pnas.032665299
	2OUZ	10.1110/ps.062729207
	2POG	10.1016/j.bmcl.2007.04.051
	2Q70	10.1016/j.bmcl.2007.06.052
	2QE4	10.1016/j.bmcl.2007.07.009
	2QXS	10.1038/nchembio.451
	2R6W	10.1073/pnas.0710802105
	2R6Y	10.1073/pnas.0710802105
	2YAT	10.1021/jm200192y
	2YJA	10.1021/ja202946k
	3DT3	10.1016/j.bmcl.2008.07.121
	3ERD	10.1016/S0092-8674(00)81717-1
	3ERT	10.1016/S0092-8674(00)81717-1
	3HLV	10.2210/pdb3q95/pdb
	3HM1	10.2210/pdb3q95/pdb
	3L03	10.2210/pdb3q95/pdb
	3OS8	10.1038/nchembio.451
	3OS9	10.1038/nchembio.451
	3OSA	10.1038/nchembio.451
	4PP6	10.7554/eLife.02057
	4PPP	10.7554/eLife.02057
	4Q13	10.7554/eLife.12792
	4Q50	10.7554/eLife.12792
	5AAV	10.1021/acs.jmedchem.5b00984
	5AAU	10.1021/acs.jmedchem.5b00984
	4XI3	Fanning SW, Mayne CG, Toy W, Carlson K, Greene B, Nowak J, Walter R, Panchamukhi S, Tajhorshid E, Nettles KW, Chandarlapaty S, Katzenellenbogen J, Greene GL, Estrogen Receptor Alpha Ligand Binding Domain in Complex with Bazedoxifene
	5FQS	10.1021/acsmedchemlett.5b00413
	5FQR	10.1021/acsmedchemlett.5b00413
	5FQP	10.1021/acsmedchemlett.5b00413
	5FQT	10.1021/acsmedchemlett.5b00413
	5FQV	10.1021/acsmedchemlett.5b00413
	5AK2	10.1021/acs.jmedchem.5b00066

Table 2
List of ESR2 (ERβ) protein IDs involved in steroid hormone -mediated signalling pathway

Sl. No.	PDB ID	Reference/DOI
	4J26	10.1021/ja311748r
	1L2J	10.1038/nsb787
	1NDE	10.1021/jm020291h
	1QKM	10.1093/emboj/18.17.4608
	1U3Q	10.1021/jm049719y
	1U3R	10.1021/jm049719y
	1U3S	10.1021/jm049719y
	1U9E	10.1021/ja047633°
	1X 76	10.1021/ja047633°
	1 X 78	10.1021/ja047633°
	1 X 7B	10.1021/ja047633°
	1 X7J	10.1016/j.str.2004.09.015
	1YY4	10.1021/jm058173s
	1YYE	10.1021/jm058173s
	1ZAF	10.1016/j.bmcl.2005.04.013
	2FSZ	10.1073/pnas.0510596103
	2GIU	10.1016/j.bmcl.2006.03.098
	2I0G	10.1021/jm060491j
	2JJ3	10.1016/j.bmcl.2007.07.009
	2NV7	10.1016/j.bmcl.2006.11.066
	2QTU	10.1016/j.bmcl.2007.08.009
	2YJD	10.1021/ja202946k
	2YLY	10.1016/j.bmcl.2011.08.041
	2Z4B	10.1016/j.bmcl.2007.06.052
	3OLL	10.1002/cbic.201000532
	3OLS	10.1002/cbic.201000532
	3OMO	10.1021/jm1011116
	3OMP	10.1021/jm1011116
	3OMQ	10.1021/jm1011116
	4J24	10.1021/ja311748r

Table 3
List of AR protein IDs involved in steroid hormone-mediated signalling pathway

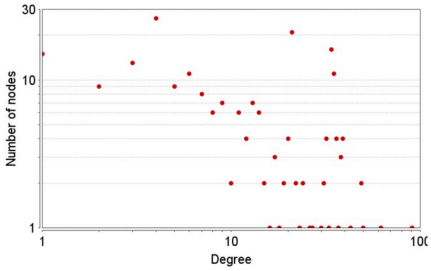
Sl. No.	PDB ID	REF/DOI
	1E3G	10.1074/jbc.M004571200
	1GS4	10.1021/JM011072J
	1T5Z	10.1074/jbc.M407046200
	1T63	10.1074/jbc.M407046200
	1T65	10.1074/jbc.M407046200
	1XJ7	10.1074/jbc.M407046200
	1XOW	10.1016/j.molcel.2004.09.036
	1XQ3	10.1016/j.molcel.2004.09.036
	1Z95	10.1073/pnas.0500381102
	2AM9	10.1110/ps.051905906
	2AMA	10.1110/ps.051905906
	2AMB	10.1110/ps.051905906
	2AO6	10.1016/j.molcel.2004.09.036
	2AX6	10.1074/jbc.M507464200
	2AX7	10.1074/jbc.M507464200
	2AX8	10.1074/jbc.M507464200
	2AX9	10.1074/jbc.M507464200
	2AXA	10.1074/jbc.M507464200
	2HVC	10.1107/S1744309106039340
	2OZ7	10.1074/jbc.M611711200
	2PIO	10.1073/pnas.0708036104
	2PIP	10.1073/pnas.0708036104
	2PIQ	10.1073/pnas.0708036104
	2PIR	10.1073/pnas.0708036104
	2PIT	10.1073/pnas.0708036104
	2PIU	10.1073/pnas.0708036104

(*continued on next page*)

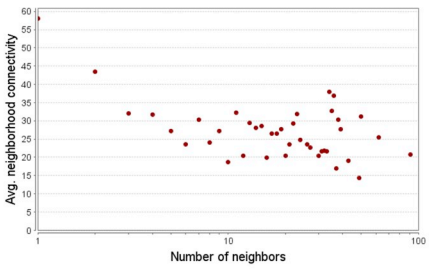
Table 3 (*continued*)

Sl. No.	PDB ID	REF/DOI
	2PIV	10.1073/pnas.0708036104
	2PIW	10.1073/pnas.0708036104
	2PIX	10.1073/pnas.0708036104
	2PKL	10.1073/pnas.0708036104
	2PNU	10.1074/jbc.M705524200
	2Q7I	10.1074/jbc.M703268200
	2Q7J	10.1074/jbc.M703268200
	2Q7K	10.1074/jbc.M703268200
	2Q7L	10.1074/jbc.M703268200
	2YHD	10.1021/jm200532b
	2YLO	10.1021/jm201098n
	2YLP	10.1021/jm201098n
	2YLQ	10.1021/jm201098n
	2Z4J	10.1107/S0907444907045702
	3B5R	10.1016/j.bmcl.2008.09.002
	3B65	10.1016/j.bmcl.2008.09.002
	3B66	10.1016/j.bmcl.2008.09.002
	3B67	10.1016/j.bmcl.2008.09.002
	3B68	10.1016/j.bmcl.2008.09.002
	3V49	10.1021/jm300249m
	3V4A	10.1021/jm300249m
	3ZQT	10.1021/jm201098n
	4HLW	10.1021/jm3015712
	3L3X	10.1074/jbc.M109.085779
	3L3Z	10.1074/jbc.M109.085779
	3RLJ	10.1021/jm2000097
	3RLL	10.1021/jm2000097
	4K7A	Liu JS, Hsu CL, Wu WG, 2014. Crystal structure of the androgen receptor ligand binding domain in complex with minoxidil
	4OEA	10.1016/j.molonc.2014.06.009
	4OED	10.1016/j.molonc.2014.06.009
	4OEY	10.1016/j.molonc.2014.06.009
	4OEZ	10.1016/j.molonc.2014.06.009
	4OFR	10.1016/j.molonc.2014.06.009
	4OFU	10.1016/j.molonc.2014.06.009
	4OGH	10.1016/j.molonc.2014.06.009
	4OH5	10.1016/j.molonc.2014.06.009
	4OH6	10.1016/j.molonc.2014.06.009
	4OHA	10.1016/j.molonc.2014.06.009
	4OIL	10.1016/j.molonc.2014.06.009
	4OIU	10.1016/j.molonc.2014.06.009
	4OJ9	10.1016/j.molonc.2014.06.009
	4OJB	10.1016/j.molonc.2014.06.009
	4OK1	10.1016/j.molonc.2014.06.009
	4OKB	10.1016/j.molonc.2014.06.009
	4OKT	10.1016/j.molonc.2014.06.009
	4OKW	10.1016/j.molonc.2014.06.009
	4OKX	10.1016/j.molonc.2014.06.009
	4OLM	10.1016/j.molonc.2014.06.009
	4QL8	10.1021/jm5009049
	5CJ6	10.1021/acs.jmedchem.5b01168

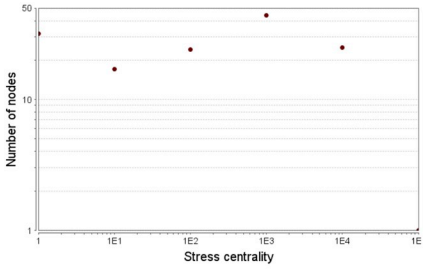
Annexure 2. Topological Parameters of PPI network of BPA-interacting proteins.



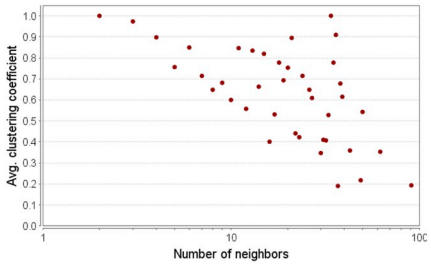
Node Degree Distribution



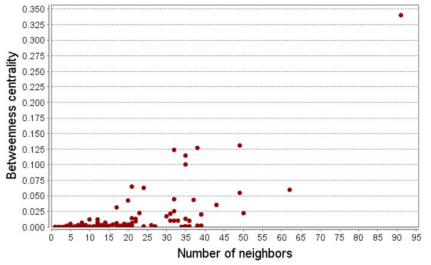
Neighborhood Connectivity Distribution



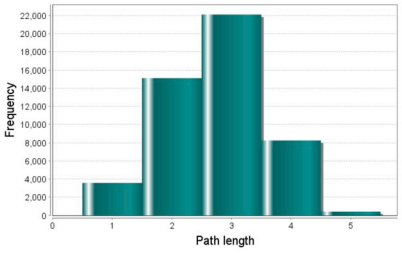
Stress Centrality Distribution



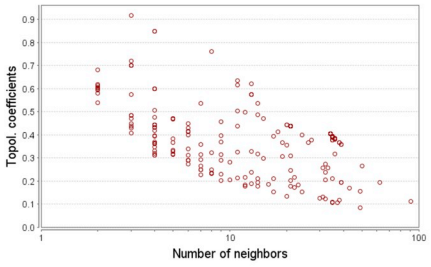
Avg. Clustering Coefficient



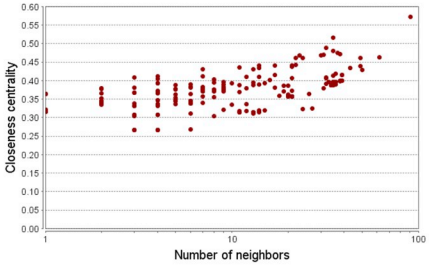
Betweenness Centrality



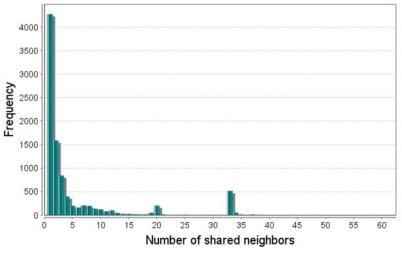
Shared Neighbors Distribution



Topological Coefficients



Closeness Centrality



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