

# Deciphering Fine Molecular Details of Proteins' Structure and Function with a Protein Surface Topography (PST) Method

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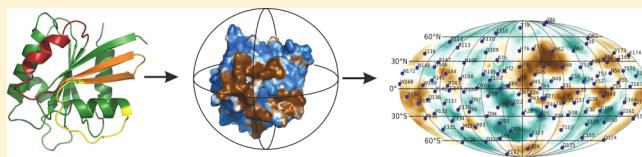
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## Supporting Information

**ABSTRACT:** Molecular surfaces are the key players in biomolecular recognition and interactions. Nowadays, it is trivial to visualize a molecular surface and surface-distributed properties in three-dimensional space. However, such a representation tends to be biased and ambiguous in case of thorough analysis. We present a new method to create 2D spherical projection maps of entire protein surfaces and manipulate with them—protein surface topography (PST). It permits visualization and thoughtful analysis of surface properties. PST helps to easily portray conformational transitions, analyze proteins' properties and their dynamic behavior, improve docking performance, and reveal common patterns and dissimilarities in molecular surfaces of related bioactive peptides. This paper describes basic usage of PST with an example of small G-proteins conformational transitions, mapping of caspase-1 intersubunit interface, and intrinsic “complementarity” in the conotoxin–acetylcholine binding protein complex. We suggest that PST is a beneficial approach for structure–function studies of bioactive peptides and small proteins.



## INTRODUCTION

The problem of intermolecular recognition is central to molecular biology, since each biochemical reaction and signal propagation event relies on interaction between two or more kinds of molecules. It is still a big challenge to discover how physicochemical properties of one molecule render those of the partner molecule, and often even availability of the spatial structure of the complex does not give a thorough understanding. At the same time, formation of a complex is governed preferentially by local characteristics of partners' surfaces, aiming to reach high “complementarity”. In such a manner, given a set of molecules of similar shape and size with known spatial structures, one can analyze global and local similarities of surface-encoded patterns, such as mobility of functional groups, shape of the surface, and distribution of charge and hydrophobicity.

Analysis of essentially two-dimensional surfaces seems to be a “reduction” as against the 3D structure. Meanwhile, a sort of reductionist's approaches are widespread in molecular and structural biology of low-molecular weight compounds and biopolymers. In the first case, quantitative structure–activity relationship (QSAR) based techniques permit construction of pharmacophore models that describe small molecules' structure–function relationships.<sup>1</sup> For proteins, a 3D > 1D “environment profile” approach converts a 3D atomistic environment of an amino acid residue in a spatial structure of a protein into a single parameter—environmental class, which is surprisingly enough for assessment of protein packing quality and identification of similar protein folds.<sup>2</sup>

Returning to surfaces, they were used to map various properties since the very origin of molecular surface conception.<sup>3</sup> Among these properties are electrostatic potential, hydrophobicity, or landscape (for example, cavities and active sites).<sup>3,4</sup> There is a handful of approaches that consider molecular surface as the specific rendezvous point for molecular species. Most of them try to identify functional areas by prediction of ligand-binding pockets,<sup>5,6</sup> sometimes taking into account physicochemical properties of the binding site.<sup>7</sup> Other methods are designed for similarity searches irrespective of protein sequences.<sup>8,9</sup> Nevertheless, protein–protein interactions are essentially beyond reach of these methods, and moreover, they do not take into account conformational flexibility of molecules. In one recent work, calculation of surface hydrophobicity is performed over the molecular dynamics (MD) trajectory to highlight conformational transition in several proteins upon phosphorylation,<sup>10</sup> but no connection with biological function was proposed.

Conventionally, these results are employed only for 3D visualization, rarely coming up to quantitative description. An exclusion is quite informative 2D maps for  $\alpha$ -helical peptides, where Kohonen networks are used to project the surface onto the torus.<sup>11–13</sup> For  $\alpha$ -helical peptides cylindrical projections proved themselves not only illustrative but also computationally relevant.<sup>14–16</sup> Another example is provided by  $\beta$ -structural three-loop toxins (e.g., cardiotoxins and neurotoxins) revealing rather flat surface, which is naturally approximated with a

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plane.<sup>17,18</sup> A “3D > 2D” approach is also realized in gnomonic projection by Chau and Dean<sup>19</sup> and spherical harmonics representation developed by Duncan and Olson.<sup>20,21</sup>

Transformation of molecular surfaces into uniform numeric arrays provides a bunch of advantages in comparison to simple visual analysis. Generally, it makes it possible to compare different surfaces and their properties in a quantitative manner and reveal common patterns or other specific features of surface organization for structurally similar objects. Calculation of hydrophobic “complementarity” in the binding pockets at the protein/ligand delimiting surface uncovers important principles in molecular recognition, and this conception may be applied to improve docking results.<sup>7,22,23</sup> Moreover, surface hydrophobicity is a good measure for conformational transitions<sup>10</sup> and generally “tags” areas of intermolecular recognition.<sup>24</sup>

However, the number of systems with simple geometry is quite limited. The question rises whether it is possible to elaborate similar approach to molecules with other than cylindrical or flat surfaces? Yes—recently, we proposed detailed spherical mapping and quantitative comparison of surface-distributed physicochemical properties for  $\alpha$ -neurotoxins from scorpions’ venom, which target voltage-gated sodium channels. This mapping revealed distinct surface patterns for neurotoxins with different channels’ selectivity,<sup>25</sup> although very similar size ( $\approx$ 65 residues), shape, secondary structure, and overall fold.

Here, we extend that approach and present a *protein surface topography (PST)* method, which is generally applicable to various classes of proteins and typical molecular modeling tasks. In particular, we focus on the following aspects: (1) delineation of common patterns and dissimilarities within a group of related proteins (here, small G-proteins); (2) visualization of conformational transitions in proteins (here, on the example of caspase-1); (3) assessment of “complementarity” of physicochemical properties at the receptor–ligand delimiting surfaces (using conotoxin—acetylcholine binding protein complexes). Major PST deliverables to the field of structural and computational biology are clear visualization of entire proteins’ surface properties as 2D-maps; account for protein dynamics; interface mapping in protein–protein complexes; tracking of conformational changes; group analysis that reveals common patterns and/or dissimilarities; improvement of quantitative robustness in docking experiments; assessment of hydrophobic and electrostatic “complementarity” at receptor–ligand binding surfaces; and more.

## METHODS

**Objects.** Several structures of small G-proteins from the adenosine diphosphate (ADP)-ribosylation factor (ARF) family were chosen for binding sites mapping and study of conformational rearrangements (Table 1). Prior to construction of molecular surface and molecular hydrophobicity potential (MHP) and electrostatic potential (ELP) calculations, all proteins were spatially aligned to the ARF6 structure in its inactive state. Conformational transition and dimer interface were demonstrated with an example of human caspase-1 (Table 1). Active and inactive states were superimposed. For illustration of ligand–receptor interactions and complementarity of physicochemical properties in the binding site, a complex of conotoxin TxIA (A10L mutant) with AchBP from *Aplysia californica* was chosen. All structures were obtained from Protein Data Bank<sup>26</sup> and are summarized in Table 1.

**Molecular Dynamics (MD) Simulations.** A dynamic map of ARF6 in the active state (Figure 1) was derived from MD

**Table 1. Overview of Objects Used**

object	PDB code	comments
ARF6	2J5X	ARF Family of G-Proteins
	1EOS	active state (GTP-bound)
	3PCR	inactive state (GDP-bound)
	3LVQ	complex with ESPG
	2W83	complex with ASAP3
	2ASD	complex with JIP4
	1J2J	complex with CTA (Cholerotoxin)
ARF1	1RRG	active state
ARL1	1R4A	inactive state
ARL1(Sc)	1MOZ	active state
ARL2	1KSG	inactive state
ARL5	1ZJ6	active state
human caspase-1	2HBQ	Human Caspase-1
	1SC1	inactive state
		active state
TxIA (A10L)	2UZ6	Conotoxins
PnIA (A10L)	2BR8	complex with <i>Aplysia californica</i> AchBP

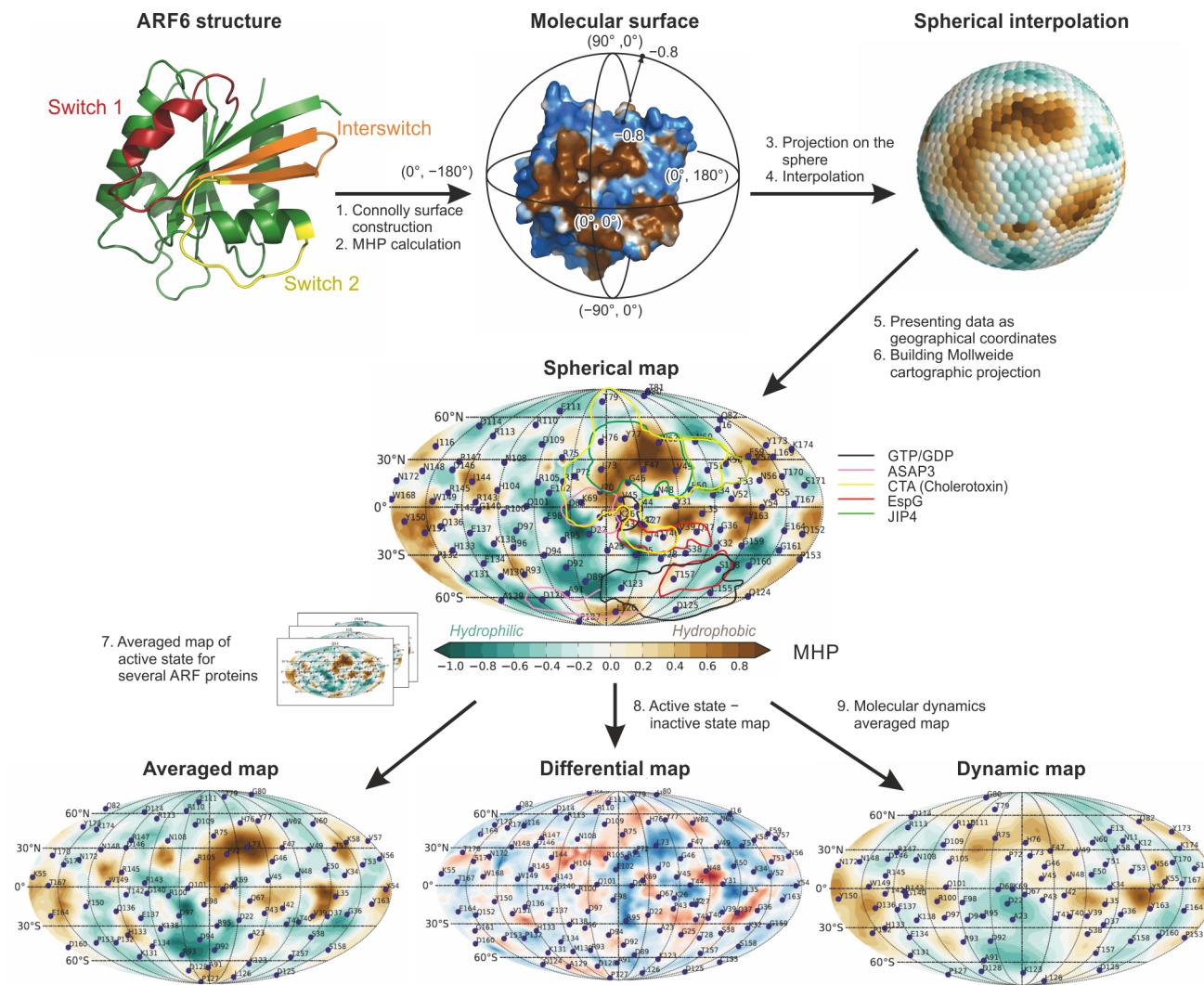
simulation, which was carried out with the Gromacs 4.0.7 package.<sup>27</sup> Briefly, a molecule was placed in rectangular water box with the necessary amount of counterions added and subjected to energy minimization, followed by heating to 300 K for 100 ps and 60 ns of unconstrained MD simulations. The Gromos96 45a3 force field<sup>28</sup> and SPC water model<sup>29</sup> were used. The trajectory was fitted to a single structure to get rid of rotation and translation. The whole protocol was taken from ref 25.

**Molecular Hydrophobicity Potential (MHP) and Electrostatic Potential (ELP) Calculations.** The molecular hydrophobicity potential approach assumes that each atom in the molecule possesses its “intrinsic” value of hydrophobicity (atomic hydrophobicity constant), according to topology of the molecule.<sup>24</sup> These constants have been determined from the database of experimental log *P* values for large number of organic compounds;<sup>30</sup> MHP at any given point is calculated as superposition of monotonically decaying with distance contributions, created by each atom. These calculations were performed with PLATINUM software.<sup>23</sup> PLATINUM was also used to create the Connolly surface of the molecule as an essential step in PST (Figure 2).

ELP calculations were performed using the adaptive Poisson–Boltzmann solver (APBS) software package.<sup>31</sup> Prior to calculation, PDB2PQR software<sup>32,33</sup> was used to assign appropriate charge and radius parameters for all molecules studied and place missing hydrogen atoms.

**Details of the PST Algorithm.** The main steps in PST (illustrated in Figure 1) are the following:

1. Calculation of the Connolly molecular surface.
2. For each dot on the surface, calculation of either MHP or ELP.
3. Projection of each dot onto a sphere. The center of the sphere was superimposed with its geometrical center of the surface, and this was set as the coordinate origin. So, a dot with coordinates ( $x, y, z$ ) may now be described by latitude =  $\arcsin(z/|r|)$  and longitude =  $\arctan(y/x)$ , where  $r$  is radius vector of the dot and latitude and longitude have the same meaning as in geography.



**Figure 1.** Overview of PST method with the example of small G-protein ARF6 in its active state. The most important structural elements of ARF6 are “switches” 1 (red) and 2 (yellow) with interswitch (orange) between them. The flowchart of the PST method is as follows: (1) Calculation of the Connolly surface of ARF6. (2) Computation of MHP value in each point of the surface. (3) Projection of each dot with its MHP value onto a sphere (concentric with the molecule). (4) Interpolation of MHP data onto a regular spherical grid. (5) Representation of all data as geographical coordinates (latitude, longitude, MHP). (6) Construction of Mollweide equal area projection of the whole protein surface. Applications: (7) Computation of an average map of G-protein active state (related ARF6, ARF1, and ARL1 molecules are used; see also Supporting Information Figure S1). (8) Differential map shows differences between active and inactive states of ARF6. (9) Molecular dynamics-averaged map for the active state of ARF6. Colored contours on the map in the center depict interaction interfaces with several effector proteins (legend is to the right).

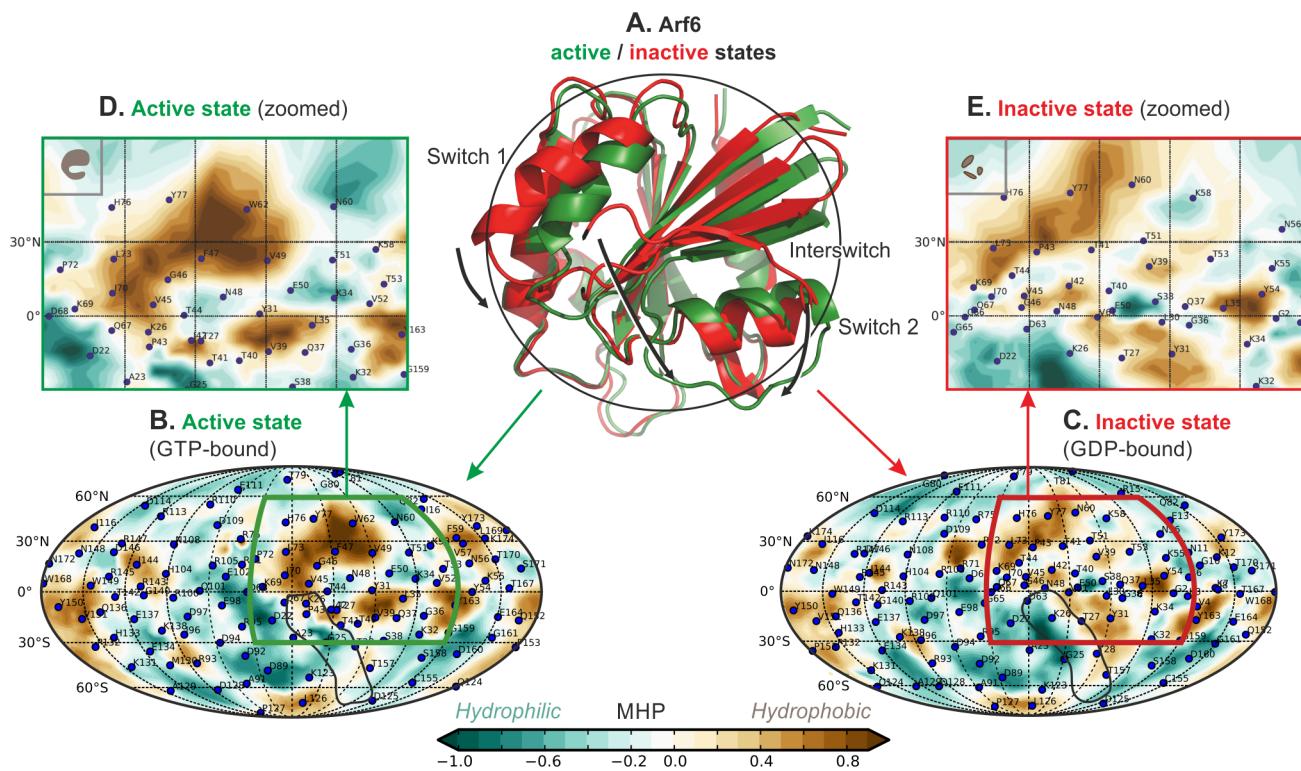
4. Interpolation of irregularly distributed data on an equal area regular grid on the sphere. For this step a sphere was represented as a polyhedron, with 3325 vertexes that form a regular, equal area grid on the sphere. Then, the MHP or ELP data was interpolated on that grid.
5. Interpolation of regular MHP data on a coordinate grid—latitude  $[-90^\circ; +90^\circ]$  and longitude  $[-180^\circ; +180^\circ]$  with  $1^\circ$  step.
6. Building of equal-area Mollweide cartographic projection<sup>34</sup> or close-up Mercator projections of a specific area.
7. Visualization of this map using color contours.

Steps 1 and 2 were performed by Platinum or APBS. Steps 3–6 were implemented in Python script with step 4 performed with the `cssgrid` module of the CSS package,<sup>35</sup> step 5, with module `scipy.interpolate.griddata` and `basemap` and step 6, with module `matplotlib`. The concept of the program code is given in the Supporting Information that is available online. Inter-

polation of data on a regular grid permits not only fancier visualization of the maps but also a variety of operations like addition, subtraction, averaging, and calculation of standard deviation that are necessary for structure–function analyses.

**Docking and Mapping of Ligand-Binding Site.** The surface of the conotoxin binding site on AchBP was mapped using PLATINUM software. The main principle is the following: (1) the surface of the ligand and the surface of receptor are constructed, (2) MHP is calculated on the receptor surface, (3) MHP data of the receptor surface is projected onto the ligand surface, (4) the ligand surface now represents hydrophobic properties of the surrounding receptor, (5) the Mollweide map is built using PST usual steps.

Protein–protein docking of TxIA (A10L) conotoxin to *A.c.* AchBP was performed by Z-dock.<sup>36</sup> MHP complementarity between receptor binding site and docked ligand surface was computed with PLATINUM, which calculates hydrophobic/hydrophilic complementarity based on surface area of favorable



**Figure 2.** Application of PST to map the active/inactive state transition. Structural rearrangement (A) between active (green) and inactive (red) states of ARF6 is reflected on the maps of hydrophobic properties of the surface (B–E). MHP values are color-coded according to the scale below. The GDP/GTP binding site is outlined with black contour in B. (C) Zoomed-in pictures of GDP/GTP binding site for active and inactive states shown in D and E, respectively. Schematic representations of hydrophobicity distribution in these regions are shown in small insets. Thick arrows in part A show conformational rearrangements between active and inactive state.

and unfavorable contacts.<sup>23</sup> For the 100 best docking solutions, MHP complementarity was calculated and analyzed together with RMSD values between each docking solution structure and that in the PDB model.

## RESULTS

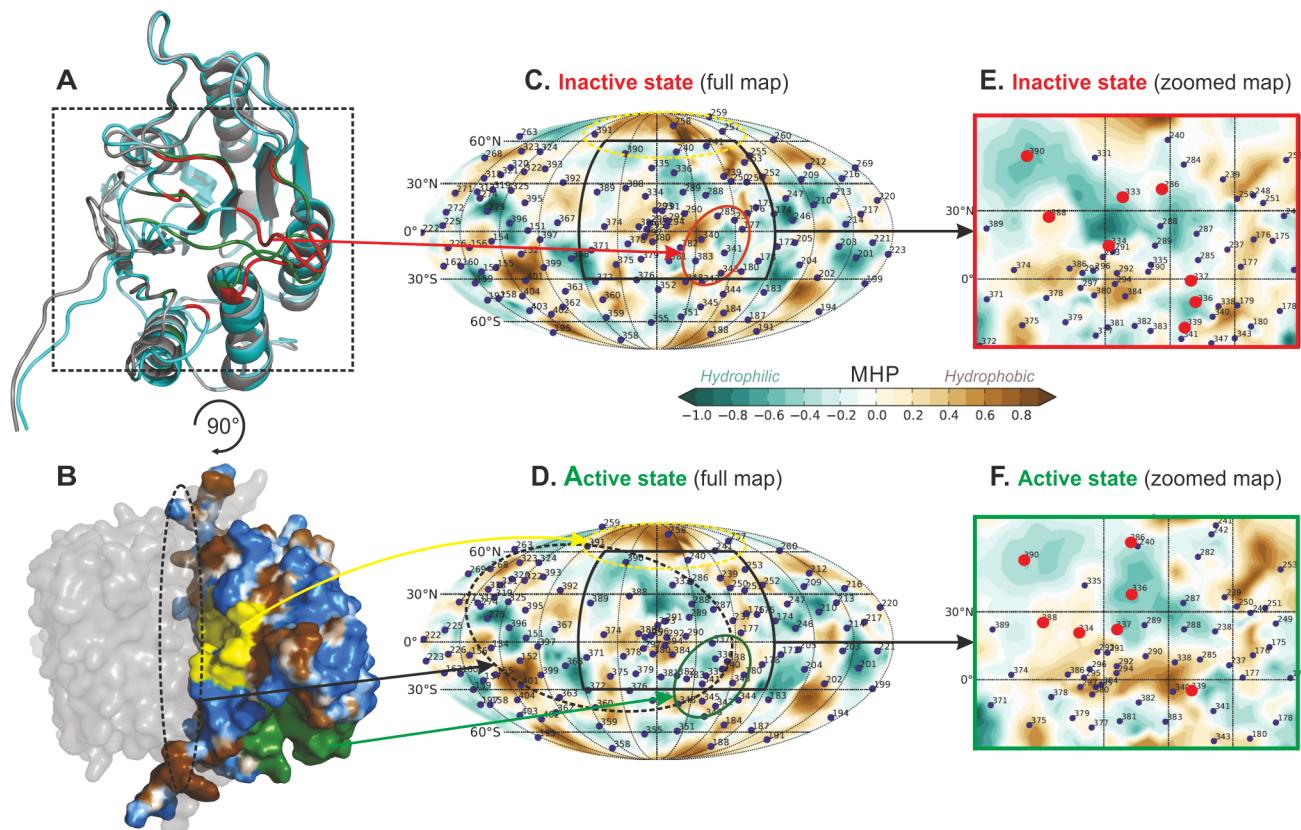
**Protein Surface Topography.** The main idea behind the PST approach is intentional simplification of complex molecular surfaces by projection onto a sphere. This permits easy visualization, direct comparison of different surfaces, and straightforward mathematical manipulations with data. Hereinafter, if otherwise is not stated explicitly, the surface-distributed property being mapped is molecular hydrophobicity potential (MHP), which is an empirical measure of hydrophobicity.<sup>24</sup> The steps of PST are represented in Figure 1 and explained in detail in Methods. The flowchart is as follows: (1) A Connolly surface<sup>3</sup> of a protein is calculated. (2) MHP, electrostatic potential, or any other parameter is calculated in each point of the surface. (3) The position of the latter one with its MHP value is projected onto a sphere, concentric with the molecule. (4) MHP data is interpolated on the regular spherical grid. (5) All data is presented as geographical coordinates (latitude, longitude, MHP). (6) A Mollweide equal area projection<sup>34</sup> of the entire protein surface is built (which is often used for sky and Earth mapping).

In order to check possible implementations of PST, we tested it on three groups of different proteins: ARF family of small G-proteins, human caspase-1, and conotoxin TxIA (A10L mutant) in complex with acetylcholine binding protein (AchBP). Both G-proteins and caspase-1 function through

switching between active and inactive states and have several binding sites for other proteins on their surface. All together, these features make them appropriate objects for illustrating PST application for studies of conformational rearrangements and mapping of active and allosteric sites. In order to check PST applicability throughout studies of receptor–ligand interactions, we applied it to the conotoxin–AchBP complex.

**Conformational Transitions and Binding Sites Mapping (Small G-Proteins).** Many proteins undergo a major conformational change that determines the molecule's function and its interaction partners. Whether two molecules will interact with each other or not is somehow encoded in their surface patterns, and conformational transition may force these patterns to emerge or disappear. Here, we apply PST methodology to illustrate the formation of specific surface patterns in active states of several ADP-ribosylation factors (ARFs; Figure 1), which are small guanine nucleotide-binding proteins (G-proteins) of approximately 20 kDa in size<sup>37</sup> that cycle between inactive (GDP-bound) and active (GTP-bound) states and selectively bind state-specific effectors.<sup>38,39</sup>

The shape and surface properties of the ARF molecule differ in active and inactive states, providing it with a regulatory capacity.<sup>40</sup> Different effector proteins recognize and specifically bind different ARF isoforms.<sup>41,42</sup> Considering that the switch regions of different ARFs have virtually identical sequences, one may ask how they implement specificity of their interaction with effector proteins. Mapping and comparative analysis of surface properties can therefore help to link conformational changes of these proteins to functional differences and effector recognition.



**Figure 3.** Surface mapping of caspase-1 in active and inactive states. (A) Structures of active (gray) and inactive (cyan) states of caspase-1 (only one monomer of each structure is shown). Residues of the active site are colored green and red, respectively. (B) Surface representation of caspase-1 dimer in the active state (one monomer is transparent gray; another colored according to MHP value). The dashed ellipse presents the dimer interface (also in D), and green and yellow surface patches represent active and allosteric sites, respectively. (C and D) PST hydrophobic maps of caspase-1 monomers (inactive and active states, respectively). (E and F) Zoomed maps of the active sites, where most structural rearrangements occur (inactive and active states, respectively). Red dots show residues involved in a series of crucial H-bonds (“hot wire”).

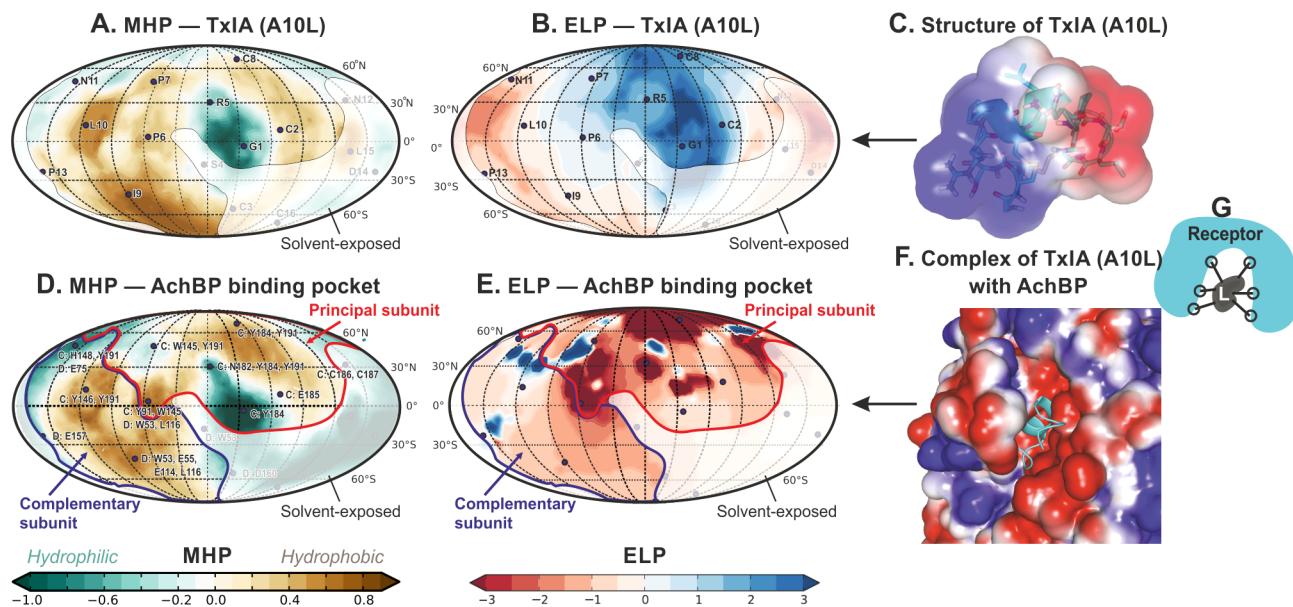
Most of the complexes between ARFs and their effectors share common features, such as similar hydrophobic and relatively flat interface (which is located in the vicinity of switch and interswitch regions; Figure 1A).<sup>43</sup> In order to have a closer look at that interface and to understand if similar structures possess similar surface properties, we analyzed all available structures of ARFs in active and inactive states using PST (Figure 1, Table 1). Maps of the active states (for four available structures) reveal a strikingly similar overall pattern with the most notable feature in the region of switch 1/switch 2 (see Figure 2b and d and Supporting Information Figure S1, left column). This common area on the map resembles a hydrophobic ring with a small hydrophilic spot inside (see inset in Figure 2d). Interestingly, active/inactive transition disrupts this similarity and results in different hydrophobic patterns on the maps of inactive states (see Figure 2c and e and Figure S1, right column). In the inactive state, the large hydrophobic patch disintegrates into several small spots (Figure 2e).

In an attempt to understand whether effector proteins bind to common ARFs area, we put projections of binding sites of several effectors on the map of the ARF6 active state (Figure 1). Residues directly involved in binding were marked on the map, thus illustrating where this interaction occurs.<sup>43–46</sup> Although our results indicate that the binding sites of effector proteins are located in various parts of the ARF6 surface, all these parts have similar hydrophobic properties among ARFs

with only two small parts that exhibit different hydrophobic properties between the two isoforms. Taken together, our experiments show that PST provides an easy and informative way for mapping several binding sites on a protein surface, as well as illustrating conformational rearrangements between different states of a protein.

**Illustrating Conformational Transition and Dimer Interface in Human Caspase-1.** Caspases are dimeric thiol endopeptidases that drive cellular processes such as apoptosis and inflammation.<sup>47</sup> Human caspase-1 is a heterodimer of tightly associated p10 and p20 subunits (Figure 3a and b).<sup>48,49</sup> Structural studies of caspase-1 reveal two conformational states: active (when the active site is occupied with substrate) and inactive (when the active site is empty or when the enzyme binds an allosteric ligand (inhibitor)).<sup>50</sup>

The question we address in this part is whether it is appropriate to apply PST for large proteins and whether the resulting map will be resolved enough to show small conformational changes that accompany an active/inactive transition and provide some insight into this spatial rearrangement (Figure 3). Mapping the dimer interface revealed that it has a symmetric pattern of a hydrophobic groove fringed with hydrophilic strands at both sides (area in black ellipse contour in Figure 3d). Such a symmetry (complementarity) of surface properties of each monomer may contribute greatly to correct and quick dimer association.



**Figure 4.** Complementarity of hydrophobic and electrostatic surface properties between binding pocket of AchBP and conotoxin. (top) Maps of hydrophobic (A) and electrostatic (B) properties of conotoxin TxIA (A10L mutant), obtained with the PST approach. Conotoxin's residues projections are shown, and the solvent-exposed area of the toxin is shaded. (C) Surface of conotoxin, colored by electrostatic potential (ELP). (bottom) Maps of the conotoxin binding pocket in AchBP (D and E). Active site residues that contact a given conotoxin residue are subscribed. Also, areas of conotoxin's contact with primary and complementary AchBP subunits are bordered with red and blue lines, respectively. (F) Complex of AchBP (colored by values of ELP) and conotoxin. (G) Schematic principle of binding pocket mapping: data from receptor surface is projected onto the ligand (L) surface, and then, PST mapping is performed.

Comparison of the maps obtained for active and inactive states of caspase-1 shows that the active site region is the only part of the protein surface where the maps significantly differ. Generally, it is more hydrophobic in the active state with respect to the inactive one. The key interaction here is a salt bridge between Arg286 and Glu390, which occurs only in the active state.<sup>50,51</sup> In the latter one, structural rearrangements bring Arg286 and Glu390 closer together, and this movement is reflected on the map as well.

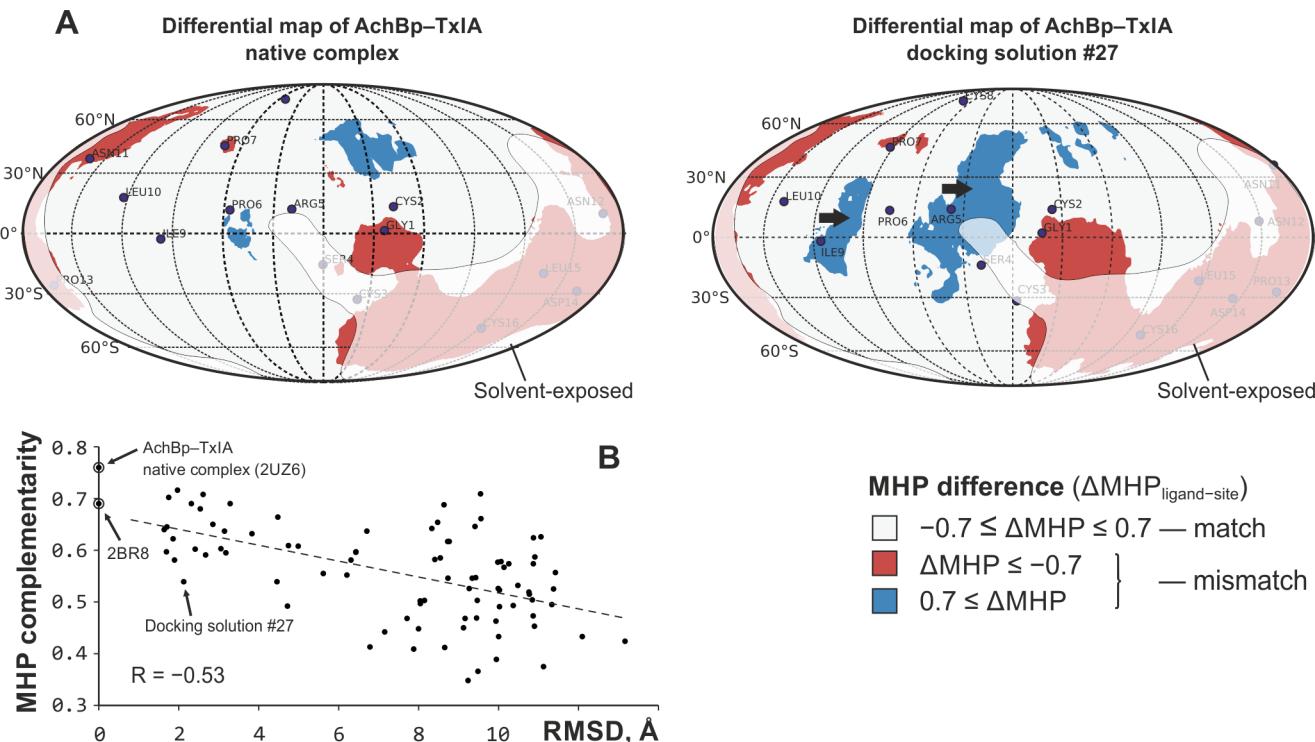
Taken together, these results prove that PST may be fruitful to study conformational transitions even in large proteins, given their relatively spherical shape. It can also illustrate complementary features of protein–protein interfaces. Analysis of the sites, depicted on the map, will be most helpful for understanding of intermolecular recognition processes.

**Match of Surface Properties at Protein–Protein Interfaces (Conotoxins–AchBP Complexes).** Along with formation of h-bonds, hydrophobic and electrostatic interactions seem to be the major factors driving intermolecular recognition and formation of biomolecular complexes. Strong attraction obviously requires a “match” (or “complementarity”) of surface-distributed properties in the area of intermolecular contact. Hydrophobic complementarity was shown to be important for molecular association,<sup>7,52</sup> and it can be pictorially illustrated by PST mapping. As an example, we have chosen conotoxins—small (12–16 a.a.) toxic peptides from venom of marine *Conus* snails. These peptides bear two or more disulfide bridges and differ in their specificity toward particular nicotinic acetylcholine receptors (nAChRs) and voltage-gated sodium, calcium,<sup>53,54</sup> and potassium<sup>55</sup> channels. Due to high selectivity to various nAChR subtypes and relative simplicity of chemical synthesis, these peptides became a useful tool in structural and functional studies of nAChRs.

Although there is currently no determined structures of nAChRs, their extracellular ligand-binding part is structurally very similar to acetylcholine binding proteins (AchBPs). Like nAChRs, the latter ones possess pentameric structure and bind conotoxins and other nAChRs ligands.<sup>56,57</sup> Given the availability of several structures of complexes between conotoxins and AchBP,<sup>56–58</sup> the latter represent an adequate model for the nAChR ligand-binding domain.

Although the discovery of AchBP was a big step toward understanding of how conotoxins recognize nAChRs, the exact binding mechanism still remains poorly understood. Here, using these known structures, we aimed to study “complementarity” between partners in the complex and describe the binding sites by mapping pocket interior on a sphere, concentric with the approximately spherical ligand. For the complex of conotoxin TxIA (A10L mutant) with AchBP,<sup>59</sup> four different maps were calculated (see Methods): maps of hydrophobic (1) and electrostatic (2) properties of a ligand (conotoxin) and maps of hydrophobic (3) and electrostatic (4) properties of corresponding binding pocket in AchBP (Figure 4). The resulting maps provide a picture of hydrophobic/hydrophilic and electrostatic match between the ligand and its binding site on the receptor. Almost the whole AchBP binding pocket is negatively charged thus allowing TxIA molecule correctly bind with its side that carries positive charges (Figure 4b and e). MHP maps reveal even stronger complementarity, so that the toxin surface exactly mirrors the surface of the receptor pocket: a hydrophilic spot in the center of the map is surrounded by more hydrophobic areas (Figure 4a and d).

To further illustrate the importance of matching of physicochemical properties at the ligand–receptor interface, we performed docking of a conotoxin TxIA (A10L) to AchBP. For each docking solution, MHP complementarity between ligand and receptor surfaces was calculated, as well as RMSD



**Figure 5.** Close-to-native protein–ligand structures exhibit high MHP complementarity. (A) Differential maps show MHP correspondence between AchBP binding site and conotoxin TxIA (A10L mutant) in native complex (left) and in one of the docking solutions (right). Maps are colored according to MHP match/mismatch (see legend below). (B) Relation between MHP complementarity in AchBP–TxIA complexes obtained in a docking run and deviation of the docking solution from reference structure (RMSD). Encircled points with RMSD = 0 correspond to crystallographic complexes (see Table 1). The arrow near the dot with RMSD  $\approx 2$  Å shows docking solution no. 27.

with native position of TxIA (A10L) (Figure 5). There is a moderate but clear anticorrelation ( $R = -0.53$ ) between MHP complementarity and RMSD (Figure 5b), so that solutions with small deviations from the correct position display higher MHP complementarity. In some cases, MHP complementarity is rather sensitive to minor changes of a ligand position inside the active site. That effect can be observed using differential maps calculated for docking solution structure and binding pocket of a receptor—deviation of only 2 Å results in a shift of large hydrophobic area on the conotoxin surface, which now does not match the hydrophobic area on the receptor surface (blue area in the center of the map, Figure 5a, right).

Summing up, PST pictorially visualizes surfaces of both a ligand and its surrounding binding site in the protein. Differential maps clearly illustrate complementarity at the receptor/ligand interface. This makes PST an appealing tool to use in docking stimulations, where additional calculation of MHP complementarity could help to sort out correct solutions.

**Identification of Membrane-Binding Pattern in Proteins.** The membrane is one of the most important compartments in the cell, and there are plenty of integral and peripheral membrane proteins. Association with the membrane most probably requires specific hydrophobic pattern at the surface of the protein, and PST should be valuable tool to predict these regions. For example, in lypoxygenase, it detects a spacious hydrophobic patch (see Supporting Information Figure S2), which corresponds to the membrane-binding surface, according to the OPM Web server.<sup>60</sup>

## DISCUSSION

### Whole-Surface Mapping of Molecular Properties.

Finding essential similarities and differences in structures of biological molecules is a grand challenge, and there are no standard techniques that can address this task for proteins—apart from small molecules, where pharmacophore-based analyses<sup>61</sup> and QSAR approaches<sup>62</sup> are being applied routinely to mine active molecules from computer databases of chemical compounds. At the same time, many peptides and bioregulators affect activity of receptors, thus representing very important class of biological objects—potential new drugs.

Nowadays, the protein–protein docking field has come of age, but it still has a number of limitations<sup>83</sup> and hardly can be applied if spatial structure of either partner is unknown or poorly determined. Given this, a method capable of revelation of similar features in protein organization is highly demanded: such a quantitative comparison of groups of molecules with different activities may shed light on functional peculiarities and give researchers a clue about biological function and possible ways of engineering of these objects. We introduce here a protein surface topography (PST) approach that utilizes molecular hydrophobicity potential (MHP) spherical projection maps (see Methods). The PST approach can be readily extended to map electrostatic potential, molecular landscape, and virtually any other surface-distributed property. Also, the method natively assesses conformational flexibility of molecules.

Projection onto a sphere is not the only possibility. The PST method may be adapted for other types of geometry: long, rod-shaped molecules may be projected onto a cylindrical surface, as it was recently implemented in our PREDDIMER Web

server.<sup>14</sup> For molecules with relatively “flat” surfaces, projection onto a plane may be a reasonable choice.<sup>17,18</sup> Many more complex molecules may require an ellipsoid projection.

Apart from group analysis, the PST method allows detailed examination of interfaces between ligand and receptor and highlights complementary surface areas (see Results), which could be of a particular interest for docking optimization or “hot-spot” search studies. The entire surface mapping is advantageous for analyzing surface rearrangement in conformational transitions as well as for displaying binding sites on the molecule’s surface. Below each of the listed applications is discussed in details.

**Group Analysis.** The basic idea of the PST approach has already proved itself useful in structure–functional studies of scorpion  $\alpha$ -neurotoxins where group analysis was successfully applied.<sup>25</sup> Quantitative presentation of molecular surface properties permitted comparative analysis of sets of small proteins in the form of group-averaged and differential maps, displaying essential similarities and differences between several groups. The main idea behind group analysis assumes that the specific function of several proteins (for example activity) results from common surface properties in key regions. Using scorpion  $\alpha$ -toxins as an example, we have shown that the averaged maps for toxins acting on mammal voltage-gated sodium channels (VGSCs) share a similar pattern of surface hydrophobicity, which is different in the case of insect VGSC-active toxins. For more details, please see the original paper.<sup>25</sup> In this article, group analysis applied to the ARF family of small G-proteins has revealed common features for all studied active states that differ significantly from corresponding inactive states (see below; Figure 2).

**Conformational Transitions.** Application of the PST approach to multistate proteins permits delineation of differences in structure and surface properties of various conformational states of a protein, as well as pictorial demonstration of allosteric and active sites. As an example of how PST could be useful in studies of such proteins, we present the ARF family of small G-proteins and human caspase-1. The conformational rearrangement follows the transition of ARFs from inactive (GDP-bound) to active (GTP-bound) states.<sup>39</sup> Mapping surface properties of both of these states shows that such a rearrangement is accompanied by distortion of surface properties. Our method has shown that active states of different ARF isoforms share the same hydrophobic pattern, responsible for binding of nonspecific effector proteins (Figures 2 and S1 (Supporting Information)). Since the active states are not distinguishable neither structurally nor by their surface properties (maps on Figures 1 and S1 are similar), one can propose that selective recognition happens with the inactive state. Indeed, in the case of ARF6 and ARF1, despite their virtually identical sequences (70% identity), inactive states feature different conformation and flexibility of pivotal switch regions.<sup>40</sup>

As was previously stated for ARF1 and ARF6, inactive states exhibit structural features that allow them to be distinguished from each other. If these specific features are the key to isoform-specific recognition by effector proteins, they would most likely be reflected on the protein surface. Our mapping method confirms that observation and expands it on ARL1 and ARL5 (Supporting Information Figure S1), providing pictorial visualization of surface and therefore conformational differences. The revealed common pattern of the active states (see Figure 1) is destroyed upon the conformational transition, and

inactive states exhibit surface properties unique to each isoform and, thus, can be recognized by isoform-specific effector proteins.

Another supporting example is caspase-1, which switches between active and inactive states upon its functioning.<sup>49,51</sup> Inspection of the X-ray structures for the active and allosterically inhibited enzyme suggests that coupling between the states may be mediated by a network of 21 hydrogen bonds formed by nine side chains of contiguous circuit of residues (“hot wire”) running from one active site through the allosteric cavity into the second active site.<sup>51</sup> Uncoupled donors/acceptors of the h-bonds may contribute to the hydrophilic area, shown on the inactive state map; whereas in the active state, these groups form h-bonds with each other, thus explaining the somewhat more hydrophobic properties of the surface in active state (Figure 3e and f). Despite the structural rearrangement occurring in a relatively small part of a protein, the difference between states can still be observed on the entire surface map (Figure 3). In summary, the PST approach is useful to analysis of how conformational transition affects physicochemical properties of proteins (including allosteric ones) and to compare these effects in several related proteins in order to find similarities and differences inside the group.

**Binding Site Characterization.** It was previously stated from known ARF complexes that effector proteins bind to the allosteric site or around it.<sup>43</sup> At the same time, surface mapping of binding sites of several regulators shows wider distribution of involved residues (Figure 1). These regions could participate in discrimination of ARF6 from ARF1 by various specific effector proteins although the extent of that difference might not be sufficient for selective recognition (Figure 1). Mapping allosteric and binding sites on the whole-surface maps permits us to obtain a holistic view of functional properties on the protein surface and offers an easy and illustrative way to analyze overlapping binding sites and their distribution on the surface.

**Interface Mapping.** A great number of biological molecules require formation of a di- or oligomeric structures to accomplish their cell functions. Blocking such association can yield nonfunctioning proteins and therefore is one of the mechanisms to regulate protein activity. In this work, we focused on the interface between monomers in caspase-1 dimer as an illustrative example of PST potentiality. Mapping this dimer interface revealed that it has a clearly visible symmetric pattern of a hydrophilic groove fringed with hydrophobic strands at the both sides (Figure 3). Such a symmetry of surface properties of each monomer might be one of the major factors determining correct and quick dimer association. In the general case, any other intermolecular interfaces (e.g., receptor–ligand, protein–DNA, protein–membrane) can be precisely characterized using the PST approach.

**Hydrophobicity and Electrostatic Potential Matching in Receptor–Ligand Complexes.** To end up with binding sites and interfaces mapping, we choose a complex between conotoxin TxIA (A10L mutant) and acetylcholine binding protein (AChBP), which is a reliable model for nicotinic acetylcholine receptor (nAChR) ligand binding domain—the natural target of conotoxins.<sup>59,64</sup> Our method shows high complementarity of hydrophobic and electrostatic properties between the toxin surface and surrounding receptor residues (Figure 4). The importance of electrostatic (mainly Arg5) and hydrophobic (Leu10) interactions was previously stated in the literature.<sup>59,64</sup> Therefore, the PST approach provides better understanding of why these residues are so important and with

which residues of the receptor they probably interact. Surface complementarity contributes greatly to the correct orientation of a ligand (conotoxin) in the binding pocket and therefore to high activity of a toxin. Thus, visualization of the ligand–receptor surfaces and subsequent calculation of hydrophobic “complementarity” in the binding pockets at the protein/ligand delimiting surface uncovers important principles in molecular recognition and might be applied to improve docking results<sup>7,22,23</sup> (Figure 5) and discover principles of molecular association.

Except being just a visualization tool, PST can be efficient upon computational design of mutant forms of ligands and/or receptor. In this case, complementarity of the contact surfaces in the predicted complexes can be easily checked in silico. As a result, the modified versions of the molecules with the highest degree of interface compatibility can be considered as promising candidates for further experimental tests.

## CONCLUSION

To summarize, the following advantages are delivered by PST to the field of structural and computational biology: (1) Analysis of large groups of molecules, where differential and group-averaged surface maps highlight similarities and differences in/between groups. (2) PST provides clear and easy visualization of conformational changes between different states of molecules or during MD. (3) Regions of particular interest such as active or allosteric sites could be marked on the whole-surface map or on “focused” enlarged maps of the specific part of the molecular surface. (4) Maps could be constructed for any property displayed on the surface (hydrophobicity, electrostatic potential, landscape, structural lability, residue variability, etc.). (5) PST could be applied in addition to existing docking techniques to improve docking performance or for general studies of ligand–receptor interactions. (6) PST provides the way for comparison of nonsimilar molecules of arbitrary size and shape: the best superposition of the maps will yield spatial alignment of molecules that could not be achieved by common spatial alignment techniques.

The main limitation of PST is the procedure of projection onto a sphere itself, since it brings distortion to the data in order to simplify it. The more a molecule deviates from the sphere, the more distorted the final picture will be, especially if one part of the molecule shields the other from the sphere surface. PST can be readily adapted for ellipsoid shape; however, more complex structures would require other ways of presenting the data. Still, the proposed method seems to be helpful for analysis of many biological molecules, their properties, and relation to biological activity.

## ASSOCIATED CONTENT

### Supporting Information

Figure S1: MHP maps of several GTP- and GDP-bound ARF proteins. Figure S2: membrane-binding pattern of lypoxygenase. Supplementary methods contain sample program code for PST in Python. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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## Notes

The authors declare no competing financial interest.

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## ABBREVIATIONS

AchBP, acetylcholine binding protein; ARF, ADP-ribosylation factors; ELP, electrostatic potential; MD, molecular dynamics; MHP, molecular hydrophobicity potential; nAChR, nicotinic acetylcholine receptors; PST, protein surface topography; VGSC, voltage-gated sodium channels

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