



## Salt-bridge dynamics in intrinsically disordered proteins: A trade-off between electrostatic interactions and structural flexibility



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### ABSTRACT

Intrinsically Disordered Proteins (IDPs) are enriched in charged and polar residues; and, therefore, electrostatic interactions play a predominant role in their dynamics. In order to remain multi-functional and exhibit their characteristic binding promiscuity, they need to retain considerable dynamic flexibility. At the same time, they also need to accommodate a large number of oppositely charged residues, which eventually lead to the formation of salt-bridges, imparting local rigidity. The formation of salt-bridges therefore opposes the desired dynamic flexibility. Hence, there appears to be a meticulous trade-off between the two mechanisms which the current study attempts to unravel. With this objective, we identify and analyze salt-bridges, both as isolated as well as composite ionic bond motifs, in the molecular dynamic trajectories of a set of appropriately chosen IDPs. Time evolved structural properties of these salt-bridges like persistence, associated secondary structural 'order-disorder' transitions, correlated atomic movements, contribution in the overall electrostatic balance of the proteins have been studied in necessary detail. The results suggest that the key to maintain such a trade-off over time is the continuous formation and dissolution of salt-bridges with a wide range of persistence. Also, the continuous dynamic interchange of charged-atom-pairs (coming from a variety of oppositely charged side-chains) in the transient ionic bonds supports a model of dynamic flexibility concomitant with the well characterized stochastic conformational switching in these proteins. The results and conclusions should facilitate the future design of salt-bridges as a mean to further explore the disordered-globular interface in proteins.

### 1. Introduction

Recent research in Intrinsically Disordered Proteins (IDPs) has brought about a paradigm shift in the central dogma of protein folding, specifically questioning the 'one sequence → one structure → one function' epitome in globular proteins [1]. IDPs lack unique three-dimensional structures under physiological conditions, but are known to carry out a multitude of important biological functions. The intrinsic disorder of a protein may be classified as i) Intrinsically Disordered Protein Regions (IDPRs) where well-defined secondary structures coexist with highly flexible disordered regions and ii) Intrinsically Disordered Proteins (IDPs) which are completely devoid of any distinct tertiary structure. The functional diversity of IDPs/IDPRs may be attributed to their potential promiscuity in binding to different structurally unrelated partners in living cells [2]. IDPs and IDPRs are usually represented as dynamic ensembles of interconvertible conformations [3] instead of well-defined structures that characterize globular proteins. The structural flexibility of IDPs/IDPRs [4] in uncomplexed form imparts higher conformational entropy [5] due to continuous

fluctuations between multiple conformers. Compared to globular proteins, IDPs and IDPRs are highly enriched in charged and polar residues and deficient in hydrophobic residues, which is denoted by a higher mean net charge to mean hydrophobicity ratio [2,6,7]. It has been observed in protamines (a class of arginine-rich IDPs with defined cellular functions) that the net charge per residue is one of the discriminating 'order' parameters responsible for the adoption of heterogeneous conformational ensembles in IDPs/IDPRs during the globule-to-coil transition [8]. Also, in contrast to the globular proteins, the hydrophobic residues are randomly scattered along the disordered sequences [4,9] that do not mediate a hydrophobic collapse [10,11] accompanied by concomitant water depletion.

Theoretical studies based on designed globular and disordered protein sequences have revealed that there is a transition of the prevalent interactions at the globular-disorder interface across the charge-hydrophobicity boundary [12]. To this end, the globular-disorder (or globule-to-coil) transition may be explained based on a compromise between hydrophobic and electrostatic interactions. The prevalence of polar and charged residues in the disordered proteins potentially

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triggers electrostatic interactions, both attractive and repulsive, which constitutes the predominant interaction in IDPs/IDPRs [13]. The role of electrostatic interactions are manifest in the binding of IDPs/IDPRs to their targets and in the binding of histone chaperones, [14] which are investigated by combined NMR and Molecular Dynamics (MD) studies [15]. One of the key cellular functions seldom observed in IDPs is their phosphorylation, initiating ultra-sensitive reactions, where the electrostatic interactions play a pivotal role, contributing to ultra-sensitivity [16]. Again, one of the major electrostatic components is the salt bridges, that comprise of ionic bonds between oppositely charged amino acids in close contact. Salt-bridges, both in isolation and as composite ionic bond motifs [17], are known to impart local rigidity in protein structures [18] (analogous to the disulphide bridges [19,20]) by bringing together distant regions in folded globular proteins, acting as molecular clips at the protein-protein interface and by promoting loop-closures as revealed in computationally modeled partially disordered proteins [17]. However, the structural plasticity of IDPs/IDPRs, rich in prolines and glycines gives rise to high coil forming propensities [4]. Hence, it would be logical to explore the dynamics of salt-bridge formation in partially/completely disordered proteins for the understanding of how IDPRs/IDPs retain structural flexibility at the expense of a large number of charged residues.

The main purpose of this study is to understand the meticulous trade-off between these two seemingly opposite phenomena which may facilitate the design of partially/completely disordered proteins. With this background, we explore the structure and dynamics of these salt-bridges, both individually and also as composite motifs in IDPs/IDPRs along their simulated MD trajectories. We analyze their dynamic persistence, their involvement in mediating secondary structural ‘order-disorder’ transitions and other associated structural features in an attempt to rationalize their paradoxical presence in good numbers in the dynamic conformational ensembles of IDPs/IDPRs. We also investigate in detail, the specific contribution of these salt-bridges in depicting the overall electrostatic interaction map of these proteins, given the importance of electrostatic interactions in IDPs/IDPRs. Furthermore, we also attempt to explain the solution-reactivity of IDPs/IDPRs from the analyses of their respective hydrophobic burial profiles.

## 2. Materials and methods

### 2.1. Selection of IDPs

To study the salt-bridge dynamics in IDPs/IDPRs, a set of four disordered proteins were chosen with varying degrees of structural disorder (ranging from 43 to 100%) in their native states, containing oppositely charged amino acids (roughly 1/3rd) ensuring the formation of salt-bridges (Supplementary Table S1). The set consisted of two partially disordered proteins (IDPRs); i) the scaffolding protein GPB from *Escherichia virus phix174*, PDB ID 1CD3, chain ID: B, ii) the human coagulation factor Xa, PDB ID: 1F0R, chain ID: B and two completely disordered proteins (IDPs), namely  $\alpha$ -synuclein ( $\alpha$ -syn) and amyloid beta (A $\beta$ 42). Amyloid beta with its exact sequence length of 42 residues (A $\beta$ 42) was chosen. Both IDPRs, 1CD3 and 1F0R consist of long contiguous stretches of disordered regions characterized by missing electron densities in their respective PDB files. These disordered stretches in IDPRs are located at the N-terminus for 1F0R, while for 1CD3 it is mainly confined to the middle regions. The sequences of the IDPs were obtained from the DISPROT database [21].

### 2.2. Atomic model building

The X-ray crystallographic structures of both 1CD3 and 1F0R (resolution: 3.5 Å & 2.1 Å and R-factor: 0.275 & 0.216 respectively) have long contiguous stretches of missing coordinates (i.e., missing electron densities) corresponding to the disordered regions. These missing disordered residues were identified by comparing the SEQRES and ATOM

records in their corresponding PDB files and cross-checked with those declared in the REMARK 465 list. The disordered regions for the partially as well as completely disordered proteins were modeled using MODELLER [22]. The modeling of the disordered regions is done in a manner such that it exactly preserves the structure of the ordered part of the protein, i.e., the RMSD (root-mean-square deviation) of the ordered part of the protein is exactly zero. Such methods were followed in earlier works [5,6].

### 2.3. Molecular dynamics simulation

Explicit-water Molecular Dynamics (MD) simulation trajectories for 1CD3, 1F0R and  $\alpha$ -syn used in the current calculation were directly obtained from a previous study [6] while, for A $\beta$ 42, an identical simulation protocol was followed [6]. The simulations were performed with AMBER 12 program [23] at  $T = 300$  K using the ff99SB force field [24,25] with periodic boundary conditions and TIP3P water model [26]. Energy minimization of each solvated protein was performed twice via the conjugate gradient method to remove unfavorable steric interactions. The energy minimized solvated protein was equilibrated in an NVT ensemble for 100 ps at an initial temperature of 100 K, while the temperature was gradually raised to 300 K at constant volume. This was followed by NPT equilibration for 5 ns at a constant temperature of 300 K and a pressure of 1 bar. An NPT production run of 100 ns with a time step of 2 fs was performed on the equilibrated system of each protein. Berendsen's temperature bath was used to maintain constant temperature with a coupling constant of 2 ps, while constant pressure was regulated by a barostat with a coupling constant of 1 ps. Trajectories were written at an interval of 2 ps, resulting in 50,000 frames (or snapshots) and all analyses were performed on the post-equilibrium 100 ns long trajectories for all four proteins.

### 2.4. Globular protein database

A database of high resolution X-ray structures of globular proteins were compiled as a reference, using the advanced search protocol of PDB [27] with the following culling criteria: (i) resolution  $\leq 2$  Å (ii) neither working nor free R-factor worse than 0.2 (iii) files containing only uncomplexed proteins without DNA/RNA/DNA-RNA hybrid, (iv) a sequence identity of maximum 30% (v) only wild type proteins including expression tags and (vi) percentage coverage of sequence 90% (Uniprot) [28]. The application of the above culling criteria resulted in 2777 unique chain entries which are mapped to 2692 PDB structures (Supplementary Dataset S1). The resulting database is referred to as GDB and was used as a benchmark to assess and evaluate certain parameters related to IDPs/IDPRs for the first time, to the best of our knowledge.

### 2.5. Secondary structural assignments

Secondary structures (helices, strands, coils etc.) were determined from the atomic coordinates by STRIDE [29] and assigned to each amino acid residue in the polypeptide chain based on the available knowledge of both hydrogen bonding pattern and the backbone geometry of existing protein structures in the PDB.

### 2.6. Identifying salt-bridges

In accordance to a recent study [17], ionic bonds within disordered proteins were detected when a positively charged nitrogen atom from the side-chains of lysine (NZ), arginine (NH1, NH2) or positively charged histidine (HIP: ND1 NE2, both protonated) were found to be within 4.0 Å of a negatively charged side-chain oxygen atom of glutamate (OE1, OE2) or aspartate (OD1, OD2).

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