

DOMAIN MOVEMENTS OF HUMAN SERUM ALBUMIN: A MULTI-DOMAIN PROTEIN IN CROWDED ENVIRONMENT

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**DEPARTMENT OF CHEMISTRY
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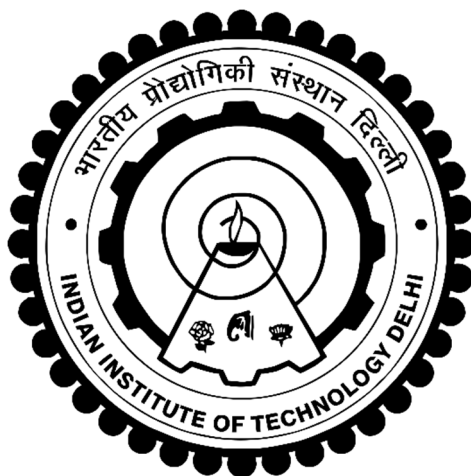
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Submitted

in fulfillment of the requirements of the degree of Doctor of Philosophy

to the



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*Dedicated to my mother and in the memory of
my father*

CERTIFICATE

This is to certify that the thesis titled “**Domain Movements of Human Serum Albumin: A Multi-domain Protein in Crowded Environment**” being submitted by **Mr. Saikat Biswas** to the Indian Institute of Technology, Delhi, for the award of degree of **Doctor of Philosophy** is a record of bonafide research work carried out by him.

Mr. Saikat Biswas has worked under my guidance and supervision and has fulfilled the requirements for the submission of this thesis, which to my knowledge has reached the requisite standard.

The results contained in this thesis are original and have not been submitted, in part or full, to any other University or Institute for the award of any other degree or diploma.

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Abstract

The thesis entitled '**Domain Movements of Human Serum Albumin: A Multi-domain Protein in Crowded Environment**' focuses on the understanding of the effect of macromolecular crowding on the domain motion of Human Serum Albumin (HSA). In prokaryotic and even more in eukaryotic cells the predominant fraction of the whole proteome belongs to the class of multi-domain proteins. The domain stabilities and the strength of inter-domain interactions in a multi-domain protein have an enormous influence on the functioning of a multi-domain protein. In this thesis we have monitored FRET based domain movement of different domain of HSA and also monitored domain specific solvation dynamics in presence of macromolecular crowder. In chapter 3 we have probed the influence of Dextran based crowding agents (Dextran 6, Dextran 40, and Dextran 70) on the relative movements of domains I and II of a multidomain protein, HSA using FRET, with Trp-214 in domain II acting as the donor and acrylodan (Ac) covalently attached to Cys-34 of domain I as the acceptor. Amongst the higher molecular weight crowders, while both Dextran 70 and Dextran 40 induced a significant decrease in the distance between the aforesaid domains, however for the latter macromolecular crowder (Dextran 40), beyond 50 g/L, no change in domain separation was observed even up to concentrations of 175 g/L. On the other hand Dextran 6 provides an asymmetric excluded volume which resulted in forced elongation of HSA along the Trp–Ac FRET axis. Based on our thermodynamic analysis we have hypothesized that for higher molecular weight crowder apart from entropic depletion forces there exist some appreciable soft interaction whereas for low molecular weight crowder excluded volume is the key factor. In chapter 4 we have provided a detailed mapping of variations in the interdomain distances (as a function of pH) in the presence of five

macromolecular crowding agents, differing based on their constituent monomers and average molecular weight(s). The influence of Dextran 6 was found to be predominantly via excluded volume, while PEG8, having a similar molecular weight, exhibited a distinct crossover from enthalpy-dominated interactions to entropy-based potentials. pH dependence of the PEG8-induced interdomain distance changes confirm the presence of a hydrophilic surface for the domain I–III side of HSA, while that for domains I and II is more hydrophobic. For the larger molecular weight crowders, the majority of the FRET distance modulation was seen to be taking place within 50 g/L crowder concentration implying significant soft interactions with HSA. Residue-level fluorescence quenching studies for the three domains coupled to the FRET results provide us further insights into the manner in which the serum protein responds to and adapts itself to varying pressure(s) of the crowded medium. In chapter 5 we have studied the solvation dynamics in aqueous solutions of covalently modified Human Serum Albumin (BADAN-HSA) in presence of increasing concentrations of macromolecular crowder of various molecular weights and shapes. The reorientation dynamics of water provides direct information on what happened to HSA when subjected to in-vivo concentration of macromolecular crowding agent irrespective of shape and size. We have also tried to correlate this slow solvation dynamics with our earlier FRET based domain movement. Our result suggests that there exist different polymer regimes in crowded solution. As evident from our result we have also observed distinct crossover from semi dilute to highly concentrated regime of crowded solution. Slow hydration dynamics of protein in presence of maximum concentration of PEG based crowder is also indicative towards glassy transition of albumin protein. In living cell the bio molecules perform their respective functions in presence of not only one type of macromolecules rather in presence of

various macromolecules with different shape and size. To mimic the intercellular environment in a better way we have used mixed macromolecular crowding and probed FRET based domain movement of HSA. In chapter 6 we have reported that the mixed crowding exerts a greater stabilizing effect than the sum of the two individual crowding agents. Detail thermodynamic study shows us the effect of macromolecular crowding is not only governed by ‘excluded volume effect’ but also on soft hydrophilic/ hydrophobic interaction. The question we have tried to address is what physical picture can be drawn upon mixing two inert macromolecules. Do they behave as an ideal solution; where by both individual effects of the entities exist or do they get merged and a new behavior arises on mixing.

'डोमेन मूवमेंट्स ऑफ ह्यूमन सीरम α ल्बुमिन: ए मल्टी-डोमेन प्रोटीन इन क्रॉडेड एन्वायरमेंट' ह्यूमन सीरम एल्बिन (एचएसए) की डोमेन गति पर मैक्रोमोलेकुलर भीड़ के प्रभाव की समझ पर केंद्रित है। प्रोकोरियोटिक और α धिक यूकेरियोटिक कोशिकाओं में पूरे प्रोटीन का मुख्य α बहुर-डोमेन प्रोटीन के वर्ग के α वर्ग आता है। बहुर-डोमेन प्रोटीन में डोमेन स्थिरता और इन्टर-डोमेन इन्टरैक्शन की ताकत एक बहुर-डोमेन प्रोटीन के कामकाज पर भारी प्रभाव पड़ती है इस थीसिस में हमने एचएसए के विभिन्न डोमेन के एफआईआरटी आधारित डोमेन आंदोलन की निगरानी की है और macromolecular crowder की उपस्थिति में डोमेन विशिष्ट सोलहशन डायनेमिक्स की निगरानी भी की है। α ध्याय 3 में हमने डेक्सट्रान आधारित भीड़ देने वाले एजेंटों (डेक्सट्रान 6, डेक्सट्रान 40 और डेक्सट्रान 70) के प्रभावों की जांच की है, एक बहुआयामी प्रोटीन के डोमेन I और द्वितीय, एचआरएस FRET का इस्तेमाल करते हुए, ट्राप -214 डोमेन II में α भिनय के साथ दाता और एसीलोडन (एसी) के रूप में स्वीकार्य के रूप में डोमेन I के सीआईएस -34 से मिलकर गलती से जुड़ा हुआ है। उच्च आणविक भार भीड़ के बीच, जबकि डेक्सट्रान 70 और डेक्सट्रान 40 दोनों उपरोक्त डोमेन के बीच की दूरी में एक महत्वपूर्ण कमी को प्रेरित किया, हालांकि बाद के मैक्रोमोलेकुलर क्रॉडर (डेक्सट्रान 40) के लिए, 50 ग्राम / एल से परे, डोमेन से जुड़ाई में कोई परिवर्तन नहीं देखा गया था यहा तक कि 175 जी / एल की सांद्रता तक दूसरी ओर Dextran 6 एक α सममित बहिष्कृत मात्रा प्रदान करता है जिसके परिणामस्वरूप टीआरपी-एसी एफआईआरटी α क्ष पर एचएसए की मजबूती बढ़ा दी गई। हमारे थर्मोडायनेमिक विश्लेषण के आधार पर हमने यह α नुमान लगाया है कि उच्च आणविक भार क्रॉडर के लिए एन्ट्रोपिक कमी बलों के α लावा कुछ सराहनीय नरम बातचीत मौजूद है जबकि कम आणविक भार क्रॉडर से बाहर किए गए मात्रा के लिए महत्वपूर्ण कारक है। α ध्याय 4 में हमने पांच मैक्रोमोलेकुलर भीड़ वाले एजेंटों की उपस्थिति में इन्टरडोमेन दूरी (पीएच के एक समारोह के रूप में) में विविधताओं के एक विस्तृत मैपिंग प्रदान की है, जो कि उनके घटक मोनोमर और औसत आणविक भार (एस) के आधार पर भिन्न हैं। डेक्सट्रान 6 का प्रभाव मुख्य रूप से बहिष्कृत मात्रा के रूप में पाया गया था, जबकि पीईजी 8, एक समान आणविक भार वाले, एथिलापी-वर्चस्व वाले इन्टरैक्शन से एन्ट्रोपी-आधारित क्षमता तक एक α लग क्रॉसओवर का प्रदर्शन किया। PEG8 प्रेरित इन्टरडोमेन दूरी परिवर्तन की पीएच निर्भरता एचएसए के डोमेन I-III पक्ष के लिए एक हाइड्रोफिलिक सतह की उपस्थिति की पुष्टि करती है, जबकि डोमेन I और II के लिए α धिक हाइड्रोफोबिक है। बड़े आणविक वजन के लिए भीड़ वाले लोगो के लिए, फ्रेट दूरी के α धिका α मॉड्यूलेशन को 50 जी / एल क्रॉवर एकाग्रता के भीतर रखा जा रहा था जो एचएसए के साथ महत्वपूर्ण

नरम बातचीत का मतलब था। रेड्यू-स्तरीय प्रतिदीप्ति, एफईआरईटी परिणाम के साथ तीन डोमेन के लिए दबाने के अध्ययनों से हमें सीरम प्रोटीन का जवाब देने के तरीके को और चर्चा प्रदान करता है जिसमें भीड़ वाले माध्यमों के लग-लग दबाव (एस) में खुद को नुकूल होता है। अध्याय 5 में हमने कई आणविक भार और आकृतियों के macromolecular crowder की बढ़ती सांद्रता की उपस्थिति में covalently संशोधित मानव सीरम Albumin (BADAN-HSA) के जलीय समाधानों में solvation गतिशीलता का अध्ययन किया है। पानी की दोबारा गतिशीलता की गतिशीलता, आकार और आकार के बावजूद macromolecular भीड़ के एजेंट की इन-विवो एकाग्रता के धीन होती है, जब एचएसए के लिए क्या हुआ पर सीधा जानकारी प्रदान करता है। हमने अपने पहले FRET आधारित डोमेन आंदोलन के साथ इस धीमी सोलहशन डायनेमिक्स को सहसंबंधित करने का भी प्रयास किया है। हमारे परिणाम से पता चलता है कि भीड़ भरे समाधानों में विभिन्न बहुलक शासन मौजूद हैं। जैसा कि हमारे परिणाम से स्पष्ट है, हमने भी समेकित समेकन भी पतले से भीड़ भरे समाधान के अधिक केंद्रित शासन को देखा है। पीईजी आधारित क्रडर की अधिकतम एकाग्रता की उपस्थिति में प्रोटीन की धीमी गति से हाइड्रेशन डायनेमिक्स एल्बुमिन प्रोटीन की चकाचौंध संक्रमण की ओर संकेत करती है। जीवित कोशिका में जैव गुण न केवल एक प्रकार के गुणों की उपस्थिति में उनके संबंधित कार्यों को करते हैं बल्कि विभिन्न आकार और आकार के साथ विभिन्न गुणों की उपस्थिति में। इंटरसेल्यूलर पर्यावरण को बेहतर तरीके से नकल करने के लिए हमने मिश्रित macromolecular भीड़ का इस्तेमाल किया है और HSA के FRET आधारित डोमेन आंदोलन की जांच की है। अध्याय 6 में हमने सूचित किया है कि मिश्रित भीड़ दोनों व्यक्तिगत जमाए जाने वाले एजेंटों की राशि के मुकाबले एक अधिक स्थिर प्रभाव डालती है। विस्तारित थर्मोडायनामिक अध्ययन से पता चलता है कि macromolecular भीड़ का असर केवल 'बहिष्कृत मात्रा प्रभाव' द्वारा नियंत्रित नहीं है बल्कि नरम हाइड्रोफिलिक / हाइड्रोफोबिक इंटरैक्शन पर भी होता है। हमने जिस प्रश्न का समाधान करने की कोशिश की है, वह है दो भौतिक गुणों के मिश्रण पर शारीरिक चित्र खींची जा सकती है। क्या वे एक आदर्श समाधान के रूप में व्यवहार करते हैं; जहां दोनों संस्थाओं के व्यक्तिगत प्रभाव मौजूद हैं या वे विलय हो जाते हैं और एक नया व्यवहार मिश्रण पर उठता है।

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