COMPUTATIONAL INVESTIGATION OF MOLECULAR CROWDING EFFECTS ON MACROMOLECULAR CONFORMATIONAL EQUILIBRIA AND SELF-ASSEMBLY

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by

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Certificate

This is to certify that the Thesis titled "**COMPUTATIONAL INVESTIGATION OF MOLECULAR CROWDING EFFECTS ON MACROMOLECULAR CONFORMATIONAL EQUILIBRIA AND SELF-ASSEMBLY**" is being submitted by **Mr. Satyendra Rajput** to the Department of Materials Science & Engineering, Indian Institute of Technology Delhi, for the award of the degree of **Doctor of Philosophy**. This Thesis is a record of bona-fide research work carried out by him under my guidance and supervision. In my opinion, his Thesis has reached the standards that fulfill the requirements of the regulations relating to the degree.

The results contained in this Thesis have not been submitted to any other University or Institute for the award of any degree or diploma.

Date:

New Delhi

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Everything comes to us that belongs to us,
if we create the capacity to receive it.

Rabindranath Tagore

Dedicated to My family,
friends, and all my well–wishers

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न्यूक्लियक् अम्लम्, प्रोटीन्, बहुपर्णिकाः, लघुचयापचयद्रव्याणि इत्यादीनां कठिनतया पैक्ड् जैव-अणुकानां उच्चसान्द्रतायाः (३०० -- ४०० ग्राम/लीटरस्य) कारणेन अन्तःकोशिकीयवातावरणं "जनसङ्ख्यायुक्तं" इति मन्यते। प्रोटीन-तञ्चन-सदृशानां प्रक्रियाणां ऊष्मागतिकी-गतिविज्ञानयोः उपरि भीड-परिवेशस्य प्रभावः अधिकाधिकं ज्ञायते। सुप्रतिष्ठिताः भीडस्य प्रभावेषु जैवअणुनां संकुचनं भवति यत् तेषां आकारस्य कारणेन संकुचकानां एन्ट्रोपिक् बहिष्कृत आयतनप्रभावैः उत्पद्यते। अद्यतन अन्वेषणैः तु सूचितम् अस्ति एतादृशेषु जटिलतन्त्रेषु मृदु ऊर्जायुक्तानां अन्तरक्रियाणां महत्त्वम्। अतः एतेषां प्रभावानां व्यापकसूक्ष्मदृष्टिकोणबोधः अद्यापि दुर्गमः एव अस्ति। अस्मिन् प्रबन्धे, अस्माभिः आणविकगतिविज्ञानस्य अनुकरणस्य उपयोगेन स्थूलअणुकानां स्वसङ्घटनस्य पतनस्य च उपरि आणविकसङ्कुलस्य प्रभावस्य अन्वेषणं कृतम् अस्ति। एतस्याः घटनायाः अन्वेषणार्थं सरलीकृतविलेयप्रतिमानानाम् विचारः कृतः, यथा कार्बनिक-कैटियन-छद्म-आइसोसायनिन्-क्लोराइड् (PIC) रञ्जकः, यः आन्तरिकरूपेण अव्यवस्थितप्रोटीनानां सदृशानि रेशेन-सदृशानि संरचनानि निर्माति, यत् प्रोटीन-सङ्ग्रहणस्य अन्वेषणं प्रदाति। आणविक-क्राउडररूपेण एथिलीन-ग्लाइकोलस्य मृदु-आकर्षक-अविशिष्ट-अन्तर्क्रियाः रञ्जक-ओलिगोमेराइजेशनं प्रतिकूलं कुर्वन्ति इति ज्ञातम्। रञ्जक-क्राउडर-आकर्षक-परस्परक्रियाभिः रञ्जक-ओलिगोमर-जलीकरण-घनत्वं न्यूनीकृतम्, येन ऊर्जावानः दण्डः उत्पन्नः यः भीड-विलयनेषु रञ्जक-अतिगुणीकरणस्य प्रतिकूलः अभवत्। अध्ययनेन स्व-संयोजक-रञ्जक-अणुषु जलीकरणे भीडस्य प्रभावः प्रकाशितः ये तेषां ओलिगोमेराइजेशनं प्रभावितं कुर्वन्ति। गहनतरं अन्वेषणं प्राप्तुं, वयं किर्कवुड-बफ-सिद्धान्ताधारित-अनुकरण-प्रयोगयोः (अस्माकं सहकारिभिः कृते) द्वयोः अपि, क्राउडर-रञ्जकयोः मध्ये प्राधान्य-अन्तर्क्रियाणां परिमाणं निर्धारयितुं सैद्धान्तिकरूपरेखां परिष्कृतवन्तः। तदनन्तरं अध्ययने वयं सामान्यजलभक्षकरेखीयशाखाबहुलकानाम् पतनसन्तुलनस्य उपरि मॉडलचार्जित-आणविक-क्राउडर-विभेदक-प्रभावानाम् परीक्षणं कृतवन्तः। पुनः बहुलकजलीकरणस्य महत्त्वपूर्णा भूमिका इति ज्ञातम्। दुर्बलतया जलयुक्ताः आयनानि रेखीय-शाखायुक्त-बहुलकयोः मध्ये भेदं कुर्वन्ति इति ज्ञातम्, येन पृष्ठसक्रिय-सदृशतन्त्रेण शाखायुक्त-बहुलकानाम् पतनं भवति। एते क्राउडराः बहुलकस्य मेरुदण्डस्य पार्श्वशृङ्खलायाः च सह महत्त्वपूर्णतया अन्तरक्रियां कृतवन्तः, येन पार्श्वशृङ्खलानां मेरुदण्डस्य च परितः जलीकरणजलस्य संरचना भिन्नप्रमाणेन बाधितवती, येन रेखीयशाखायुक्तबहुलकानाम् पतनस्य भिन्नाः प्रवृत्तयः अभवन्। अन्ते, अस्माभिः एतेषां निष्कर्षाणां प्रमाणीकरणार्थं $16-$अवशेष $\beta-$हेयरपिनपेप्टाइडस्य संरचनात्मकप्राथमिकतासु आभारित-क्राउडर-प्रभावेषु अस्माकं अन्वेषणं विस्तारितम्। आदर्श आयनिक-क्राउडर्-इत्यनेन पृष्ठसक्रिय-सदृश-तन्त्रेण अधिकानि पतितानि गैर-देशीय-पेप्टाइड्-संरचनानि प्रवर्धितानि, येन पेप्टाइड-अवस्थानां अनफोल्ड्-समूहस्य आभारित-आणविक-क्राउडर-प्रकारस्य प्रति संवेदनशीलतां सूचयति परन्तु कथियनिक-अनायनिक-

क्राउडराः न प्राप्ताः पेप्टाइडस्य गुटितसमूहं महत्त्वपूर्णतया प्रभावितं कर्तुं। समग्रतया अस्मिन् शोधप्रबन्धे प्राप्ताः परिणामाः जैव–आणविक–उपरि आणविक–सङ्केत–प्रभावानाम् उदयमान–दृष्टिकोणं रेखांकयन्ति संरचना स्वसभा च। निष्कर्षाः प्रकाशयन्ति यत् जैव–आणविक–पतनस्य प्रभावं ज्ञातुं भीड–प्रणालीषु मृदु–आकर्षक–अन्तर्क्रियाणां लेखाकरणस्य आवश्यकता वर्तते। कार्येण अपि दर्शितं यत् आकर्षकं सह आणविक–सङ्घटनम्अ न्तरक्रियाः रञ्जकानां अल्पसंख्य–कीकरणं प्रतिकूलं कर्तुं शक्नुवन्ति। यत्र, आभारित–आणविक–क्राउडराः बहुलक–वास्तुकलायां भेदं कृत्वा बहुलकानाम् परितः जलीकरण–संरचनायाः आधारेण तेषां पतनस्य नियमनं कर्तुं शक्नुवन्ति। निष्कर्षाणां निहितार्थाः सन्ति यत् आन्तरिकरूपेण अव्यवस्थितप्रोटीनानां मुक्त ऊर्जा परिदृश्यानां आकारं दातुं भीडस्य भूमिका, जैव अणुनां द्रव–द्रवचरणपृथक्करणाय आभारित–भीड़–वातावरणानां कृते तथा च अनुरूप–अनुप्रयोगानाम् कृते प्रतिक्रियाशील–बहुलक–आधारित–सामग्रीणां डिजाइनं कर्तुं च।

सार

कोशिका के भीतर का वातावरण, न्यूक्लिक एसिड, प्रोटीन, पॉलीसैकेराइड और छोटे मेटाबोला–इट्स जैसे सघन रूप से पैक किए गए जैव–अणुओं की उच्च सांद्रता (300–400 ग्राम/लीटर) के कारण "भीड़भाड़ वाला" माना जाता है। प्रोटीन वलन जैसी प्रक्रियाओं की ऊष्मागतिकी और गतिकी पर भीड़ भरे वातावरण के प्रभाव को तेजी से पहचाना जा रहा है। भीड़ के सुस्था–पित प्रभावों में उनके आकार के कारण भीड़ के एन्ट्रोपिक बहिष्कृत आयतन प्रभावों के कारण जैव–अणुओं का संघनन शामिल है। हालाँकि, हाल की जाँचों ने ऐसी जटिल प्रणालियों में नरम, ऊर्जावान अंतःक्रियाओं के महत्व का संकेत दिया है। इसलिए, इन प्रभावों की व्यापक सूक्ष्म समझ अभी भी अप्राप्य है। इस थीसिस में, हमने आणविक गतिशीलता सिमुलेशन का उपयोग करके मैक्रोमोलेक्यूल्स के स्व–संयोजन और पतन पर आणविक भीड़ के प्रभावों का पता लगाया है। इस परिघटना की जांच करने के लिए, सरलीकृत विलेय मॉडलों पर विचार किया गया, जैसे कि कार्बनिक धनायनिक स्यूडोआइसोसायनिन क्लोराइड (पीआईसी) डाई, जो आंतरिक रूप से अव्यवस्थित प्रोटीन के समान तंतु–जैसी संरचनाएं बनाती है, तथा प्रोटीन एकत्रीकरण के बारे में अंतर्दृष्टि प्रदान करती है। यह पाया गया कि आणविक क्राउडर के रूप में एथिलीन ग्लाइकॉल की नरम–आकर्षक, गैर–विशिष्ट अंतःक्रियाएं डाई ऑलिगोमेराइजेशन को प्रतिकूल रूप से प्रभावित करती हैं। डाई–क्राउडर आकर्षक अंतःक्रियाओं ने डाई ऑलिगोमर्स के जल–योजन घनत्व को कम कर दिया, जिसके परिणामस्वरूप ऊर्जावान दंड उत्पन्न हुआ, जिसने क्राउडेड विलयनों में डाई ऑलिगोमेराइजेशन को प्रतिकूल रूप से प्रभावित किया। अध्ययन में स्व–संयोजन डाई अणुओं के जलयोजन पर भीड़ के प्रभावों पर प्रकाश डाला गया, जो उनके ओलिगोमेराइजेशन को प्रभावित करता है। गहन अंतर्दृष्टि प्राप्त करने के लिए, हमने किर्कवुड–बफ सिद्धांत पर आधारित सिमुलेशन और प्रयोग (हमारे सहयोगियों द्वारा किए गए) दोनों से क्राउडर्स और रंगों के बीच अधिमान्य अंतःक्रियाओं को मापने के लिए सैद्धांतिक ढांचे को परिष्कृत किया। इसके बाद के अध्ययन में, हमने जेनेरिक हाइड्रोफोबिक रैखिक और शाखित पॉलिमरों के पतन संतुलन पर मॉडल आवेशित आणविक क्राउडर्स के विभेदक प्रभावों की जांच की। एक बार फिर पाया गया कि बहुलक जलयोजन ने महत्वपूर्ण भूमिका निभाई। यह पाया गया कि कमजोर रूप से जलयोजित ऋणायन रैखिक और शाखित बहुलकों के बीच विभेद करते हैं, तथा सर्फेक्टेंट जैसी क्रियाविधि के माध्यम से शाखित बहुलकों के पतन को प्रेरित करते हैं। ये क्राउडर बहुलक की रीढ़ और पार्श्व श्रृंखलाओं दोनों के साथ महत्वपूर्ण रूप से अंतःक्रिया करते हैं, जिससे पार्श्व श्रृंखलाओं और रीढ़ के चारों ओर जलयोजन जल की संरचना विभिन्न स्तरों पर बाधित होती है, जिसके परिणामस्वरूप रैखिक और शाखित बहुलकों के पतन की विभिन्न प्रवृत्तियां उत्पन्न होती हैं। अंत में, हमने इन निष्कर्षों को मान्य करने के लिए $16-$अवशेष $\beta-$हेयरपिन पेप्टाइड की संरचनात्मक प्राथमिकताओं पर आवेशित क्राउडर्स के प्रभावों पर अपनी जांच को आगे बढ़ाया।

मॉडल एनायनिक क्राउडर्स ने सर्फेक्टेंट–जैसी क्रियाविधि के माध्यम से अधिक संकुचित गैर–देशी पेप्टाइड संरूपणों को बढ़ावा दिया, जो आवेशित आणविक क्राउडर्स के प्रकार के लिए पेप्टाइड अवस्थाओं के अनफोल्डेड समूह की संवेदनशीलता को दर्शाता है। हालाँकि, यह पाया गया कि धनायनिक और ऋणायनिक क्राउडर्स पेप्टाइड के फोल्डेड समूह को महत्वपूर्ण रूप से प्रभावित नहीं करते हैं। कुल मिलाकर, इस थीसिस में प्राप्त परिणाम जैव–आणविक संरचना और स्व–संयोजन पर आणविक भीड़ के प्रभावों के उभरते दृष्टिकोण को रेखांकित करते हैं। निष्कर्ष इस बात पर प्रकाश डालते हैं कि जैव–आणविक पतन पर प्रभाव को समझने के लिए भीड़–भाड़ वाली प्रणालियों में कोमल, आकर्षक अंतःक्रियाओं को ध्यान में रखना आवश्यक है। इस कार्य से यह भी पता चला कि आकर्षक अंतःक्रिया वाले आणविक क्राउडर रंगों के ऑलिगोमियराइजेशन को प्रतिकूल रूप से प्रभावित कर सकते हैं। जबकि, आवेशित आणविक क्राउडर बहुलक संरचना के बीच भेदभाव कर सकते हैं और बहुलकों के चारों ओर जलयोजन संरचना के आधार पर उनके पतन को नियंत्रित कर सकते हैं। इन निष्कर्षों में आंतरिक रूप से अव्यवस्थित प्रोटीनों के मुक्त ऊर्जा परिदृश्यों को आकार देने, जैव–अणुओं के द्रव–द्रव प्रावस्था पृथक्करण के लिए आवेशित भीड़ भरे वातावरण और अनुरूप अनुप्रयोगों के लिए उत्तरदायी बहुलक–आधारित सामग्रियों को डिजाइन करने में भीड़ की भूमिका के निहितार्थ हैं।

Abstract

Intracellular environment is considered as crowded due to the high concentrations (300 – 400 g/L) of tightly packed biomolecules such as nucleic acids, proteins, polysaccharides and small metabolites. The impact of crowded milieu on thermodynamics and kinetics of the processes like protein folding is being increasingly recognized. The well-established effects of crowding include the compaction of biomolecules caused by entropic excluded volume effects of crowders due to their size. Recent investigations, however, have indicated the significance of the soft, energetic interactions in such complex systems. Therefore, a comprehensive microscopic understanding of these effects remains elusive. In this thesis, we have explored the effects of molecular crowding on the self-assembly and collapse of macromolecules using molecular dynamics simulations. To investigate this phenomena, simplified solute models were considered, such as the organic cationic pseudoisocyanine chloride (PIC) dye, which forms fibril-like structures similar to intrinsically disordered proteins, offering insights into protein aggregation. It was found that the soft-attractive, non-specific interactions of ethylene glycol as a molecular crowder disfavoured dye oligomerization. The dye-crowder attractive interactions reduced the hydration density of dye oligomers, leading to an energetic penalty that disfavored dye oligomerization in crowded solutions. The study highlighted the effects of crowding on the hydration of the self-assembling dye molecules that influence their oligomerization. To gain a deeper insights, we refined the theoretical framework to quantify the preferential interactions between crowders and dyes, from both simulations and experiments (performed by our collaborators) based on Kirkwood-Buff theory. In the subsequent study, we examined the differential effects of model charged molecular crowders on the collapse equilibria of generic hydrophobic linear and branched polymers. It was once again found that the polymer hydration played a crucial role. Weakly hydrated anions were found to discriminate between linear and branched polymers, inducing the collapse of branched polymers through a surfactant-like mechanism. These crowders interacted significantly with both the backbone and side chains of the polymer, disrupting the structure of hydration waters around the side-chains and backbone to different extents, leading to different propensities of collapse of linear and branched polymers. Finally, we extended our investigation into the effects of charged crowders on the structural preferences of a 16−residue

$\beta-$hairpin peptide to validate these findings. The model anionic crowders promoted more collapsed non-native peptide conformations via surfactant-like mechanism, indicating the sensitivity of the unfolded ensemble of the peptide states to the type of charged molecular crowders. However, the cationic and anionic crowders were found not to significantly affect the folded ensemble of the peptide. Overall, the results obtained in this thesis underscore the emerging view of molecular crowding effects on biomolecular structure and self-assembly. The findings highlight that soft, attractive interactions in crowded systems need to be accounted for in order to understand the effects on biomolecular collapse. The work also showed that molecular crowders with attractive interactions can disfavour oligomierization of dyes. Whereas, the charged molecular crowders can discriminate between polymer architecture and regulate their collapse based on the hydration structure around the polymers. The findings have implications for the role of crowding in shaping the free energy landscapes of intrinsically disordered proteins, charged crowded environments for liquid-liquid phase separation of biomolecules and for designing responsive polymer-based materials for tailored applications.

Permissions

Permissions have been taken from the respective journals for the publications related to the work presented in this thesis.

List of Publications Related to Work Presented in this Thesis as of Date of Submission of Thesis.

1. **S. Rajput**, R. Pollak, K. Huber, S. Ebbinghaus, D. Nayar, "Ethylene glycol energetically disfavours oligomerization of pseudoisocyanine dyestuffs at crowded concentrations", *Soft Matter*, 2023, **19**, 6399 − 6413.

2. **S. Rajput**, D. Nayar, "Eects of polymer architecture and charged molecular crowders on hydrophobic polymer collapse", *ACS Polym. Au* 2024, **4**, 289-301.

3. **S. Rajput**, Sibasankar Panigrahy, D. Nayar, "In silico view of crowding: Biomolecular processes to nanomaterial design", *ACS Omega*, 2024, **9**, 28,
29953 − 29965.

4. L. Koch, **S. Rajput**, D. Nayar, S. Ebbinghaus, K. Huber, *Self-Assembly of Pseudo Isocyanine Chloride in the Presence of Attractive Polyethylene Glycol Crowders. J. Phys. Chem. B*, 2025.

5. **S. Rajput**, D. Nayar *Anionic Crowders Strongly Influence $\beta-$Hairpin Peptide Unfolded Conformations Crowders Impact on $\beta-$Hairpin Structure.* **(Under Peer−review)**

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ABBREVIATIONS

MD	Molecular Dynamics
RDF	Radial distribution function
IDP	Intrinsically disordered protein
PES	Potential energy surface
LLPS	Liquid-Liquid phase separation
REMD	Replica Exchange Molecular Dynamics
SA	Simulated Annealing
AMD	Accelerated Molecular Dynamics
SMD	Steered Molecular Dynamics
KBI	Kirkwood-Buff Integral
R-KBI	Running-Kirkwood-Buff Integral
K-KBI	Krüger-corrected Kirkwood-Buff Integral
G-KBI	Ganguly-corrected Kirkwood-Buff Integral
B-KBI	Both-corrected Kirkwood-Buff Integral
EG	Ethylene glycol
PEG	Poly-ethylene glycol
DP	Degree of polymerization
Poly-I	Linear polymer
Poly-II	Branched polymer
SASA	Solvent-accessible surface area
PMF	Potential of mean force
RMSD	Root Mean Square Deviation

SYMBOLS NOTATION

$W_s(r)$	Depletion potential
V_{ov}	Overlap volume
$\Gamma_{23}(r_p)$	Preferential binding coefficient of the co-solute (3) on solute (2) molecules
k_B	Boltzmann constant
R	Gas constant
T	Temperature
Λ_{db}	de Broglie wavelength
V^b	Biased potential
V^u	Unbiased potential
G	Gibbs free energy
$G_{ij}(r)$	Kirkwood-Buff integral
g_{ij}	Radial distribution function
$w(r)$	Potential of mean force
$\Delta G^{u\to b}$	Change in free energy from unbound to bound state
$\Delta E_{dv}^{u\to b}$	Change in enthalpy from unbound to bound state
$\Delta S_{dv}^{u\to b}$	Change in entropy from unbound to bound state
a_{33}	Derivative of $\ln a_3$ (activity of crowders) w.r.t. crowders concentration (c_3)
μ_{23}^M	Chemical potential derivative of component 2 (PIC dyes) as a function of component 3 (EG) concentration
Γ_{23}^A	Preferential binding coefficient of EG to dye molecules in the aggregated (A) state
Γ_{23}^D	Preferential binding coefficient of EG to dye molecules in the dispersed (D) state
μ^*	Excess chemical potential
ϕ	Interaction energy between solute (polymer)-solvent (comprising crowder and water)
$\Delta G^{E\to C}$	The change in free energy of polymer collapse

$\Delta S_{pv}^{E\to C}$	Total entropy change during the transition from extended (E) to collapse (C) state
$CR+$	Positively charged crowders
$CR-$	Negatively charged crowders
R_{g}	Radius of gyration
$\omega_i^b(R_{\mathrm{g}})$	Harmonic bias potential
k_b	Force constant
$R_{g,i}^{\mathrm{ref}}$	Desired equilibrium value of the reaction coordinate
N_c	Number of the crowders
R_g^{core}	Radius of gyration of the core residues
q_{tet}	Tetrahedral order metric