1. **Name of Faculty Member**: Dr. Rishu Jain

2. **Designation:** Assistant Professor

3. **Contact details:**

Office: Department of Chemistry, Gujranwala Guru Nanak Khalsa College, Ludhiana

Phone No.: 0161-521198

Residence: #82, Delta City, Malerkotla Road, Alamgir, Ludhiana, 141122

Mobile: 7696066484

Email: rshjn334@gmail.com

4**. Area of specialization**: Biophysical Chemistry

5. **Awards / Honours / Fellowships**:

- Selected as ‘‘INSPIRE FELLOW’’ by Department of Science and Technology. This fellowship is awarded to the first rank holders for doctoral research in any university in INDIA, Innovation in Scientific Pursuit for Inspired Research (INSPIRE), A new scheme of THE GOVERNMENT OF INDIA, offers standing opportunity through fellowships at the level same as NATIONAL ELIGIBILITY TEST qualified candidates.
- UGC NET (June 2010) qualified with all India rank 77th.
- University Gold Medallist in M.Sc Chemistry with distinction.
- Awarded college color by Govt. College Malerkotla for excellent academic record in B.Sc (Medical).
- Awarded a trophy by Bharat Vikas Prishad for first position in School in Matric.

6. List of best publications:

- Factor Defining the Effects of Glycine Betaine on the Thermodynamic Stability and Internal Dynamics of Horse Cytochrome *c*, **R Jain**, D Sharma, S Kumar, R Kumar, Biochemistry 53 (32), 5221-5235, 2014.

- Effects of alcohols on the stability and low-frequency local motions that control the slow changes in structural dynamics of ferrocytochrome *c*, **R Jain**, D Sharma, R Kumar, The Journal of Biochemistry 154 (4), 341-354, 2013.

- Role of macromolecular crowding and salt ions on the structural-fluctuation of a highly compact configuration of carbonmonoxycytochrome *c*, R Kumar, D Sharma, **R Jain**, S Kumar, Biophysical chemistry 207, 61-73, 2015

- Guanidine hydrochloride-induced alkali molten globule model of horse ferrocytochrome *c*, **R Jain,** S Kaur, R Kumar, The Journal of Biochemistry 153 (2), 161-177, 2012.

- Analysis of the pH-dependent stability and millisecond folding kinetics of horse cytochrome *c*, **R Jain**, R Kumar, S Kumar, R Chhabra, MC Agarwal, Archives of biochemistry and biophysics 585, 52-63, 2015.

- Viscosity-dependent structural fluctuation of the M80-containing Ω-loop of horse ferrocytochrome *c*, R Kumar**, R Jain,** Chemical Physics 418, 57-64, 2013.

- Structural, kinetic and thermodynamic characterizations of SDS-induced molten globule state of a highly negatively charged cytochrome *c*, **R Jain**, D Sharma, R Kumar, R Kumar, The Journal of Biochemistry 165 (2), 125-137, 2018.

- Effects of lyotropic anions on thermodynamic stability and dynamics of horse cytochrome c, **R Jain,** MC Agarwal, R Kumar, D Sharma, R Kumar, Biophysical chemistry 240, 88-97, 2018

7. Highlights of research work:

To find out the effects of alcohols on the low-frequency local motions that controls slow changes in structural dynamics of native-like compact-states of proteins, the effects of alcohols on structural fluctuation of M80-containing Ω-loop have been evaluated by measuring the rate coefficients for slow thermally-driven CO-dissociation reaction of a natively-folded carbonmonoxycytochrome *c* (NCO) under varying concentrations of alcohols (methanol, ethanol, 1-propanol, 2-propanol, 3°-butanol, 2,2,2-trifluoroethanol (TFE)) at pH 7.0. As alcohols concentration is increased within the subdenaturing limit of denaturant, the rate coefficients for CO-dissociation reaction decrease, indicating that subdenaturing concentrations of alcohols decrease the spatial displacement of thermal motions of Ω-loop. The spatial displacement of thermal motions of the Ω-loop is decreased most for TFE and 1-propanol and least for methanol. This finding indicates that the thermal motion of the protein in the subdenaturing limit of alcohols is controlled by the hydrophobicity of alcohol as well as by some specific effects of alcohols. Thermal denaturation studies of ferrocytochrome *c* (Ferrocyt *c*) and myoglobin (Mb) at pH ~7.0 and lysozyme (Lyz) at pH ~2.3 in the presence of various concentrations of these alcohols suggest that alcohols decrease the thermal stabilities of native and partially denatured proteins. The stabilization free energy ($\Delta\Delta G$) of Ferrocyt *c* and Lyz in alcohols solution was calculated from the slope of the Wyman-Tanford (WT) plot and water activity. The *m*-values obtained from the slope of $\Delta\Delta G$ *vs* [alcohols]

plots were found more negative for longer and linear chain alcohols, consistent with destabilization of proteins by alcohols through the disturbance of hydrophobic interactions and hydrogen-bonding.

Compatible osmolyte such as glycine betaine (GB) and low concentrations of chaotropic denaturants such guanidine hydrochloride (GdnHCl) and urea decrease the motional freedom of

native Ferrocyt *c* at pH 7.0. This deduction is made from the kinetic and thermodynamic parameters measured for CO-dissociation reaction of NCO under varying concentrations of GB, GdnHCl and urea at pH 7.0. Measurement of the rate coefficients for CO-replacement reaction of carbonmonoxymyoglobin (MbCO) by hexacyanoferrate ions under varying concentrations of GB at pH 7.0 suggests that GB also restrict the internal dynamics of native Mb. The rate coefficients and activation thermodynamic parameters (activation enthalpy and activation entropy) measured for CO-dissociation reaction of NCO under varying concentrations of GdnHCl and urea in the absence and presence of 1.0 M GB at pH 7.0 indicate that (i) within the subdenaturing limit of denaturants, GB and GdnHCl or urea show a cumulative effect on the constrained dynamics of NCO, and (ii) en route from subdenaturing to denaturing conditions large-scale subglobal unfolding motions come to dominate the dynamics and the inclusion of GB opposes the structural fluctuations that cause unfolding of the protein. Thermal- and chemical denaturation studies of ferricytochrome c (Ferricyt *c*), Ferrocyt *c* and Mb at pH 7.0 in the presence of different concentrations GB and TMAO at pH 7.0 and at pH 3.8-4.5 suggest that GB and TMAO increase the thermodynamic stability of these proteins at neutral pH, while decrease it at mildly acidic pH. Thermodynamic analysis of thermal and urea-induced unfolding transitions of Ferricyt *c* and Mb measured at different GdnHCl concentrations in the absence and presence of GB or TMAO at pH 7.0 and pH 3.8-4.5 suggests that GB and TMAO counter the deleterious effect of denaturant in native proteins at neutral pH while they show the additive effect on the destabilizing action of denaturant at mildly acidic pH 3.8-4.5.

To determine the effect of chaotropic and kosmotropic salts on the low frequency local motions that controls slow changes in structural dynamics of native proteins, the rate coefficients and activation thermodynamic parameters (activation enthalpy and activation entropy) for slow thermally driven CO association reaction of native Ferrocyt *c* have been measured under varying concentrations of salts (NaCl, NaBr, NaI, Na_2SO_4, $NaNO_3$, and

$NaClO_4$) at pH 7.0. At low to intermediate concentrations, the ions dissociated from both chaotropic and kosmotropic salts decrease the rates of CO association while they increase the activation enthalpy and activation entropy for it. This finding suggests that the low concentrations of chaotropic and kosmotropic ions restrict the internal dynamics of native Ferrocyt *c* (i) by electrostatic screening of the protein charges, and (ii) by lowering the conformational entropy of proteins through binding interactions. At relatively higher concentrations, the chaotropic ions modulate the internal dynamics of native proteins according to Hofmeister series (ClO_4^-> I^-> NO_3^-> Br^-). Thermal and chemical denaturation studies of native Cyt *c* and Mb at pH 7.0 and acid-denatured Lyz at pH 2.3 in the presence of various concentrations of these salts suggest that kosmotropic salts increase the thermodynamic stability of the native proteins at pH 7.0 while the chaotropic salts decrease it at pH 7.0 but increase it at pH 2.3. Furthermore, the effect of these salts on the thermodynamic stability of these proteins follow the Hofmeister series (SO_4^{2-}> Cl^- > Br^- > NO_3^- > I^- > ClO_4^-). The stabilization free energy ($\Delta\Delta G$) for Ferrocyt *c* in salts solution was calculated from the slope of the WT plot and water activity. The *m*-values were also estimated from the slope of $\Delta\Delta G$ versus [salts] plots. The *m*-value was found to be most negative for $NaClO_4$ and least for Na_2SO_4, consistent with stabilization of proteins according to the Hofmeister series (SO_4^{2-}> Br^- > NO_3^- > I^- > ClO_4^-).

This thesis also evaluates the effects of chaotropic denaturants (GdnHCl and sodium dodecyl sulfate (SDS)) on the stability and dynamics of native and base-denatured proteins. Comprehensive spectroscopic (CD, fluorescence, NMR) studies suggest that the low concentrations of GdnHCl (≤ 0.2 M) and SDS (≤ 0.4 mM) transform the base-denatured carbonmonoxycytochrome *c* (Cyt-CO) and Ferricyt *c* to molten globule (MG) states, respectively. Single Trp59 fluorescence experiments show that the interactions of $GdnH^+$ ions dissociated from GdnHCl (≤ 0.2 M) or Na^+ ions dissociated from SDS (≤ 0.2 mM) cause the molecular compaction in the base-denatured proteins. The near-UV CD spectra for base denatured protein in the presence of GdnHCl (≤ 0.2 M) and SDS (≤ 0.4 mM) suggest that the GdnHCl (≤ 0.2 M) and SDS (≤ 0.4 mM) induced MG-states of base-denatured protein have disordered tertiary structures. The far-UV CD spectra for base denatured protein in the presence of SDS (≤ 0.4 mM) reveal the formation of substantial content of secondary structures in the SDS-induced MG-state of base-denatured protein. Kinetic experiments involving the measurement of the CO association reaction with alkaline Ferrocyt *c* at pH 12.6 and native Ferrocyt *c* at pH 7.0 in the presence of different concentrations of GdnHCl or SDS

indicate that the low concentrations of GdnHCl (≤ 0.2 M) or SDS (≤ 0.2 mM) restrict the internal dynamics of the native and base denatured proteins. Thermodynamic analysis of the thermal denaturation curves of base-denatured Ferricyt *c* at pH 12.8 (±0.2) and native Ferricyt *c* at pH 7.0 measured in the presence different concentrations of GdnHCl (near-UV CD at 282 nm) and SDS (far-UV CD at 222 nm) indicate that the low concentrations of GdnHCl and SDS increase the thermal stability of base-denatured protein while decrease the thermal stability of native protein.

This thesis work also analyzes the effects pH on the thermodynamic stability and folding dynamics of proteins over the pH range from 3.0 to 13.0. Thermodynamic analysis of the thermal and chemical denaturants (GdnHCl and urea)-induced unfolding transitions of Ferricyt *c*, Ferrocyt *c* and Lyz measured at several different pH values, ranging from pH 3.0 to pH 13.0 reveals that the Ferricyt *c* and Ferrocyt *c* have maximum thermodynamic stability between the pH 8.0 and pH 9.5 while the Lyz has maximum thermodynamic stability at pH~4.0. Theoretically predicted electrostatic unfolding energies of Ferricyt *c* and Lyz over the pH range from pH 0.0 to pH 14.0 also reveal that Ferricyt *c* and Lyz are maximally stable at pH 8.0-9.0 and pH 4.0, respectively. Unfolded Ferrocyt *c* in refolding buffer at pH 7.0 and pH 12.7 refolds rapidly to native-state. Between pH 7.0 and pH 12.7, the activation free energy barrier for folding of Ferrocyt *c* varies by less than 1.0 kcal mol^{-1} while the folding free energy for Ferrocyt *c* which is measured by two-state analysis of GdnHCl-induced unfolding transitions of Ferrocyt *c* varies more than 9.0 kcal mol^{-1}. This finding indicates that the large disparity in thermodynamic, stability of protein between pH 7.0 and pH 12.7 is not strongly reflected in the refolding rates. The classic Wyman-Tanford linkage relation was used to calculate the β^{pH}-value for folding of Ferrocyt *c*, which is less than 0.1 between pH 7.0 and pH 12.7, indicating that the electrostatic interactions are weakly formed in the transition state and exhibit a very small effect on the folding kinetics.

Keywords: Constrained dynamics, *m*-value, water activity, Wyman-Tanford plot, counteraction, cumulative effect, chaotropic salts, kosmotropic salts, molten globule, entropic stabilization, folding kinetics, pH-effect, electrostatic interactions.