

Columbia University
Chemical Engineering Department
Sanat Kumar

DOE Award # : DE-SC0007019

Name of the recipient (Institution): Sanat Kumar; Chemical Engineering, Columbia University

Project title: Optimizing immobilized enzyme performance in cell-free environments to produce liquid fuels.

Name of the PI: Sanat Kumar

Date of the report: February 5, 2015

Research period covered by the report: September 1, 2011 – August 30, 2014

Effort Certification

Investigator	Role	Time Funded	Effort
Sanat Kumar	PI	9/11-8/14	1 month/year (8.33% effort)
Mithun Radhakrishna	Grad student	9/11-8/14	100%
Dan Zhao	Grad Student	9/11-8/13	10% (helped with some experiments)
Joseph Moll	Grad Student	7/13	5% (preliminary experiments carried on Zhao)

Proposal Goals

The overall goal of this project was to optimize enzyme performance for the production of bio-diesel fuel. Enzyme immobilization has attracted much attention as a means to increase productivity. Mesoporous silica materials have been known to be best suited for immobilizing enzymes. A major challenge is to ensure that the enzymatic activity is retained after immobilization. Two major factors which drive enzymatic deactivation are protein-surface and inter-protein interactions. Previously, we studied protein stability inside pores and how to optimize protein-surface interactions to minimize protein denaturation. In this work we studied the effect of surface curvature and chemistry on inter-protein interactions. Our goal was to find suitable immobilization supports which minimize these inter-protein interactions. Our studies carried out in the frame work of a Hydrophobic-Polar (HP) model showed that enzymes immobilized inside hydrophobic pores of optimal sizes are best suited to minimize these inter-protein interactions. Besides, this study is also of biological importance to understand the role of chaperonins in protein disaggregation. Both of these aspects profited immensely with collaborations with our experimental colleague, Prof. Georges Belfort (RPI), who performed the experimental analog of our theoretical works. Several of our results were published in a series of joint publications.

Progress and Results

We set to discover mechanisms through which protein aggregation can be mitigated. We placed a hydrophobic surface at $z=0$ and an athermal surface at $z_{\max}$ (systems studied with $z_{\max}=43$ and $z_{\max}=20$) and varied the value of surface hydrophobicity (λ) of the surface at different temperatures. We report four interesting findings.

1. The number of inter-protein contacts decreases with an increase in surface hydrophobicity.
2. There is a drastic decrease in the number of inter-protein contacts at a particular value of λ corresponding to the adsorption of the protein onto the surface. To rationalize these findings we note that at very low values of λ , the protein has no propensity to adsorb onto the surface and hence inter-protein attractions in the bulk dominate. For values of $\lambda > \lambda_c$ (λ_c corresponds to the adsorption transition of the protein) the protein surface interaction energy dominates over inter-protein interactions and hence there is a drastic decrease in the number of inter-protein contacts.
3. The number of inter-protein contacts at any given value of surface hydrophobicity (λ) increases with decrease in temperature. At higher temperatures, the chain entropy is the dominant force and hence there are fewer inter-protein contacts. As the temperature is decreased, energy dominates over entropy and consequently both the inter-protein interaction and the protein-surface interaction increases. **(Figure 1)**
4. Finally, at any given temperature and surface hydrophobicity the number of inter-protein contacts increase as the distance between the confining flat walls decreases, which shows the effect of concentration on protein aggregation. **(Figure 2)**

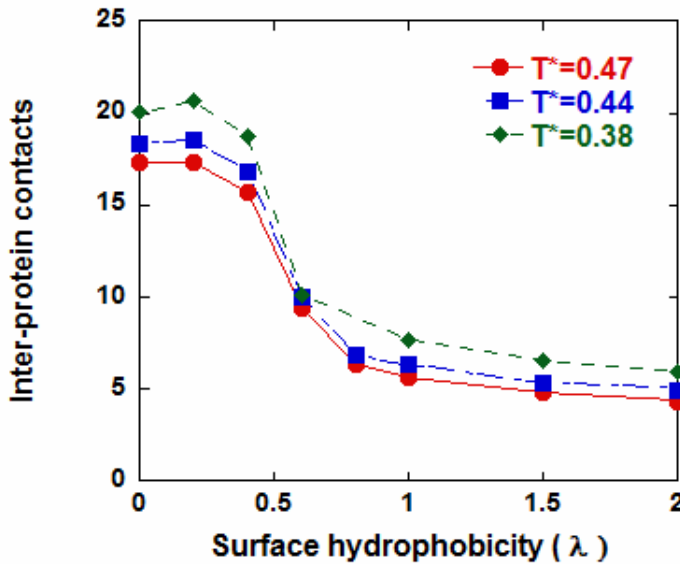


Figure 1) Average number of inter-protein contacts as a function of surface hydrophobicity (λ) of two 42mer model proteins at different temperatures (T^*)

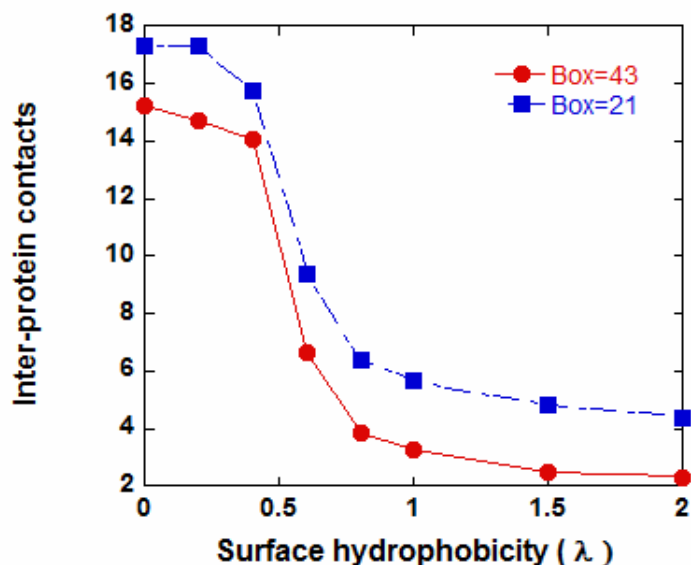


Figure 2) Average number of inter-protein contacts of the two 42mer model proteins as a function of surface hydrophobicity at $T^*=0.47$, at two different wall separations.

Now we study the effect of curvature (positive, zero and negative) on protein aggregation. **Figure 3** shows the behavior of the 42mer two protein system under different geometries at $T^*=0.47$. The effect of curvature on the percentage loss of the native contacts with varying surface hydrophobicity (λ) at low temperature (near the folding temperature) is shown in **Figure 4**. All the above geometries (except radius =4) were considered under the same volume (approximately 8000 lattice sites). The results can be rationalized as below.

- a) Adsorption on the inside (negative curvature) of a cavity: The adsorption of a protein inside a small athermal cavity ($R=4$) results in an increased number of inter-protein contacts compared to that on a flat surface. This is simply a confinement effect, which results in an increased protein concentration. As the value of λ is gradually increased we observe a decrease in inter-protein contacts due to an increase in protein-surface interactions. Because very strong protein surface interactions are necessary to break inter-protein interactions, the percentage loss in the native contacts is highest in the case of this smallest pore ($R=4$) relative to any other, larger size cavity.

Adsorption inside larger cavities ($R=12$) proves this point. Since inter-protein interactions are less favored, we do not need such high surface binding to break these inter-molecular associations. Consequently, fewer native contacts are lost relative the $R=4$ case, especially for intermediate values of λ ($1.0 < \lambda < 1.5$). For higher values of λ ($\lambda > 1.5$), as expected, we lose native contacts due to protein-surface interactions.

Comparing our results for $R=4$, 12 and a flat surface, we observe that for large enough λ at $R=12$ there are no inter-protein contacts; the loss of native contacts are also minimized under these conditions. While a more detailed study of different radii would allow us to pinpoint the optimal size more precisely (we have tried different radii upto $R=15$, but our results are dominated by simulation uncertainties), it is apparent that cavity confinement

has an optimal size where the loss of native contacts are minimized, while inter-protein interactions are completely absent. Presumably, these inter-protein interactions are exchanged in favor of protein-surface interactions. This combined behavior might be the mechanism by which chaperonins disaggregate and then refold proteins.

- b) Adsorption on the outside of a cavity: An increase in surface hydrophobicity results in decreased inter-protein contacts due to surface adsorption, as discussed above. Adsorption on the outside of small cavities ($R=4$) cannot completely mitigate inter-protein interactions due to the proximity of the adsorbing protein chains. Instead, adsorption on the outside of larger cavities ($R=9$) helps to mitigate inter-protein interactions due to increased center to center distance between the adsorbed proteins as shown by previous experiments. Note that, while adsorption on the outside seems to track the behavior inside cavities, the behavior is always a little worse. We conjecture that this arises because the threshold energy for adsorption is higher in the “outer” case.

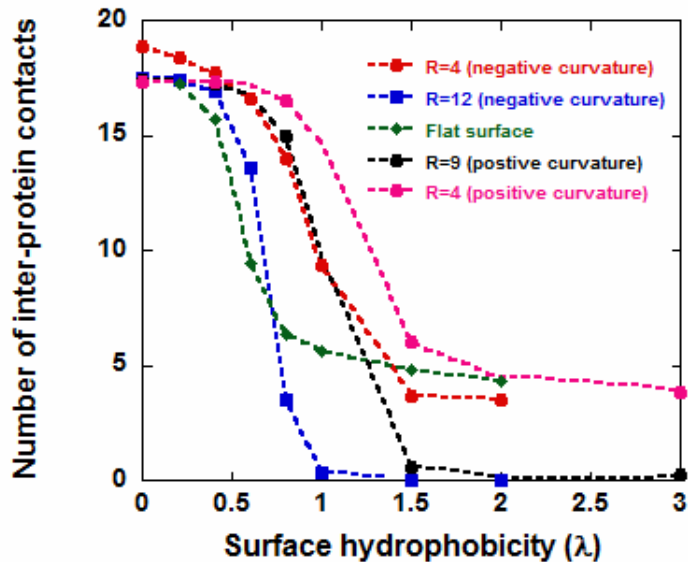


Figure 3: Average number of inter-protein contacts of the two 42mer model proteins as a function of surface hydrophobicity (λ) at $T^*=0.47$ in different geometries.

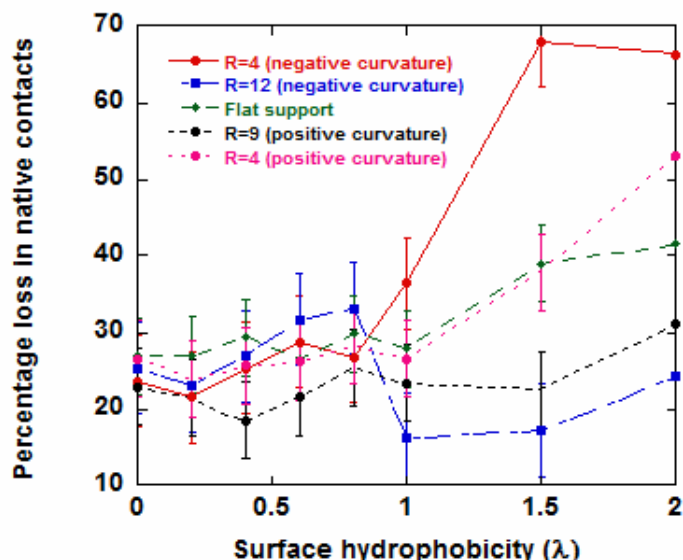


Figure 4: Percentage loss in the number of native ‘H-H’ contacts of the two 42mer model proteins as a function of surface hydrophobicity (λ) in different geometries at $T^*=0.36$

A list of papers (mark as published, in press, or submitted) in which DOE support is acknowledged.

1. **Radhakrishna, M.;** Kumar, S. K., Surface mediated protein disaggregation, *Langmuir* 2014, 30, 3507.

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2. **Radhakrishna, M.;** Grimaldi, J.; Belfort, G.; Kumar, S. K., Stability of Proteins Inside a Hydrophobic Cavity. *Langmuir* 2013, 29, 8922

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3. **Radhakrishna, M.;** Sharma, S.; Kumar, S. K., Enhanced Wang Landau sampling of adsorbed protein conformations. *J Chem Phys* 2012, 136, 114114.
4. *The authors want to thank Dr. Thomas Wust for providing the specific heat data for benchmark HP sequences. M.R. wants to thank Dr. Behnaz Bozorgui for her inputs*

regarding the method. ***The authors want to thank the Department of Energy (Grant No. DE-FG02-11ER46811) for funding.***