PROGRAMME BOOKLET

In House Symposium

April 21, 2018

Programme Booklet

Department of Chemical Engineering
Indian Insitute of Science, Bangalore 560012

A warm Welcome from CEA...

Welcome to the 2018 Chemical Engineering In-House Symposium at the Indian Institute of Science. This event is primarily meant to highlight the cutting edge research being conducted by students and research staff of the Chemical Engineering Department, and is in continuance of a long tradition in the department.

The 2018 In-House Chemical Engineering Symposium is jointly organized by the Chemical Engineering Association (CEA) and the Chemical Engineering Department. We thank the Chairman of the department, Prof. Ganapathy Ayappa, who is a constant source of inspiration and support to CEA.

We have an exciting line-up of presentations this year. These presentations demonstrate the diversity of research being conducted in the department and include areas like rheology, molecular dynamics, catalysis, energy storage, biological systems engineering and medical diagnostics, among others. The event will feature 14 oral presentations and 21 poster presentations from our students. In addition, Dr. Banani Chakraborty, a faculty member in the department, will deliver a lecture on DNA nanotechnology.

Every year, this symposium is enthusiastically attended by several of the department's alumni. We anticipate several of our alumni from industry to be present this year as well, which will create excellent networking opportunities. This is also a wonderful opportunity for our students to learn about all the exciting research happening within the department and I hope that this will lead to fruitful collaborations.

Finally, it would be remiss of me not to mention the efforts of all student members of CEA, who have worked really hard to make this event a success.

I really hope that you enjoy this event...

Bhushan J. Toley
President
Chemical Engineering Association

Technical Program

8:30 - 8:55	Registration and Coffee	
8:55 - 9:00	Chariman's address	
	Session I	
9:00 - 9:15	M Rajasekharan	The Structure, Dynamics and Relaxation of Water Confined in Graphene Oxide Slit Pores
9:15 - 9:30	Sushant Kumar	Paper Based Non-Enzymatic Glucose Sensor
9:30 -9:45	Peter Dsouza	Origin of secondary flows in Sheared Granular Materials
9:45 - 10:00	Anil Chilmula	Water Gas Shift Reaction Over Ionic Substituted Manganese Oxide Catalysts
10:00 - 11:30	**Coffee and Poster Session I**	
	Session II	
11:30 - 12:15	Faculty lecture by Dr. Banani Chakraborty	Origami based functional DNA nanotechnology and bio sensing through DNA aptamers
12:15 - 12:30	Jatin Panwar	Fusible alloy microelectrodes integrated microfluidic impedance cytometry for cell-in-droplet quantification
12:30 - 12:45	Dharitri Rath	Imbibition and partial saturation in paper-based microfluidic devices
12:45 - 2:00	**Lunch & Photo Session**	
	Session III	
2:00 - 2:15	Saurabh Umrao	Protein-induced fluorescence enhancement as aptamer sensing mechanism for thrombin detection
2:15 - 2:30	Sita Kalyani	A simulation study of Homogeneous Nucleation of molecular solids
2:30 - 2:45	Rubesh Raja	Mathematical Model unravels the orchestration of lasting viremic control upon early bNAb therapy in SHIV infection
2:45 - 3:00	Amar Garg	Stochastic simulations of affinity maturation in germinal centers suggest optimal passive immunization protocols
3:00 - 4:30	**Coffee and Poster Session II**	
	Session IV	
4:30 - 4:45	Amanuel Gebrekrstos	Process mediated polymorphism, crystallographic texture and structure-property correlation in crystalline/amorphous blends
4:45 - 5:00	Disha Jain	Steam Methane Reforming over Cobalt Titanates
5:00 - 5:15	Amit Behera	Molecular Dynamics Simulations reveal the role of membrane cholesterol during pore forming pathway of Cytolysin A
5:15 - 5:30	Abhishek Ranade	Coffee Powder aided Uniform Palladium Deposition on Silver Nanowires
5:30 - 5:45	**Prizes and vote of thanks**	
5:45 onwards	**High Tea**	

Poster Session I

P1	Sathishkumar N	Highly Sensitive Point-of-Care Immunoassays based on paper microfluidics
P2	Neha Lamba	Non-catalytic synthesis of fatty acid methyl esters (FAMEs) using supercritical fluids
P3	Bhanupriya Boruah	Photocatalytic properties of immobilized $AgBiO_3$ on cellulose acetate membrane for bacteria inactivation and 4-Nitrophenol degradation
P4	Debayan Das	Uniform rehydration of a sample fluid on dried paper membrane using microfluidic distributor
P5	Subhasish Baral	Modelling how reversal of immune exhaustion elicits cure of chronic hepatitis C after the end of treatment with direct-acting antiviral agents
P6	Poornima Ramamohan	Lattice Boltzmann Simulation of Nanoparticles in a Lamellar Phase
P7	Utkarsh Sinha	Droplet-in-Drop structure in Agitated Dispersions
P8	Md. Aslam Ansari	Harnessing natural convection in redox flow batteries: Proof of concept
P9	Khantesh Agrawal	Printed Electrodes for Polymer Electrolyte Membrane Fuel Cells
P10	Kaustubh Badwekar	Mass Transport from Walls of Soft Micro Channels
P11	Ananthu James	HIV Evolution in Transmission Potential Landscape

Poster Session II

P12	Mithlesh Meena	
P13	Pramita Sen	Modelling synergy between anti-HIV drugs
P14	Ravi Kumar Reddy	A method to calculate interfacial tension at solid-liquid interface
P15	Shivanand Kumar	A Robust Thermodynamic Theory for Gas Hydrates
P16	Surbhi Kumari	Antifreeze proteins – a molecular dynamics study
P17	Priyanka V	Isothermal Droplet Digital Quantification of Nucleic Acids
P18	Satyaghosh Maurya	Investigating oligomerization pathways of ClyA pore forming toxin
P19	Vaseef Rizvi	Translation to replication switching by resource segregation during *Flavivirus* life cycle
P20	Prithiv Natarajan	Serially diluted droplet generation for nucleic acid quantification
P21	Navjot Kaur	Paper-based microfluidics for rapid and low-cost DNA testing

Abstracts…

Coffee Powder aided Uniform Palladium Deposition on Silver Nanowires

Abhishek Ranade and S Venugopal

Palladium (Pd) is a commonly used sensing element in many room-temperature hydrogen sensors, as it can absorb large
amounts of hydrogen to form palladium hydride (PdH_x), which causes changes in its electrical and physico-chemical properties. However, the use of pure palladium (Pd) as sensing element for hydrogen (H_2) g as leads to structural deformation and hysteretic response over the desired range of operation. Alloying Pd with metals like Nickel (Ni), Silver (Ag), Gold (Au), Platinum (Pt) etc. can reduce such undesirable effects. In particular, silver-palladium (Ag-Pd) alloy show increased sensitivity and faster response, compared with other alloys [1]. Our objective was to develop a simple process for fabricating silver-palladium nanostructures on flexible substrates for monitoring pipeline leaks at ISRO's rocket launching facility. For this, we envisaged the use of a toning step to uniformly coat palladium onto silver nanostructures obtained using a Print – Expose – Develop method developed in our group. Initially, palladium (Pd) toning was carried out using a solution of palladium chloride ($PdCl_2$) and citric acid ($C_6H_8O_7$), based on a recipe available in the silver-halide photographic literature [2]. However, this resulted in a non-uniform deposition of palladium (Pd) on the silver nanostructures, which was similar to structures obtained using direct Galvanic exchange of silver by palladium. We discovered that the use of a commercially-available coffee powder (BRU^{TM} instant) led to a uniform coating of palladium on top of the silver nanostructures. In this talk, I will present the results of our studies on fabricating silver-palladium nanostructures on flexible substrates.

References:

[1] Knapton, A. G. "Palladium alloys for hydrogen diffusion membranes." Platinum Metals Review 21.2 (1977): 44-50

[2] King, Sandy. "The Kallitype Process"

Process mediated polymorphism, crystallographic texture and structure-property correlation in crystalline/amorphous blends

Amanuel Gebrekrstos, Giridhar Madras, Maya Sharma[1], Satyam Suwas[1], Sumit Bahl[2] and Suryasarathi Bose[2]
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Specific interactions between the blend components (especially in crystalline/ amorphous blends) induce miscibility under certain conditions However, depending on the processing history and the composition, the crystalline component crystallizes in different polymorphs. Herein, the effects of the amorphous content and various processing conditions on the phase transformation and the resulting texture were systematically assessed in blends of polyvinylidene fluoride and poly(methyl methacrylate). To this end, various blends were prepared by melt mixing and were subjected to different processing conditions like rolling, poling, and rolling followed by poling. To assess the different polymorphs of the crystalline component (polyvinylidene fluoride) on account of the processing history, spectroscopic, X-ray diffraction, thermal, mechanical and segmental transitions were systematically investigated and an attempt was made to correlate the observations with the resulting texture in the blends. The crystallographic texture was evaluated using X-ray diffraction and the mechanical properties as a result of the texture were studied by dynamic mechanical analyzer. The spectroscopic techniques revealed predominantly α phase in as-pressed samples, irrespective of the amorphous content However, interestingly, after rolling, the blends showed predominantly β phase. More interestingly, the highest β phase content ($> 95\%$) was obtained for blends that were rich in the crystalline phase which were deformed up to a strain of 80%. It was observed that higher the strain, lower the relaxation leading to stronger texture and facilitated the formation of β polymorph. This phase transformation after rolling was accompanied by an increase in the degree of crystallinity and storage modulus. The dielectric response revealed that the samples that were initially rolled followed by poled showed maximum dielectric constant and low dielectric loss as compared to the rolled or compression molded samples.

This study evidently demonstrates that various processing conditions and the content of the amorphous phase significantly influenced to β transition, crystallographic texture and mechanical properties and the dielectric response of the blends. Moreover, the mechanical relaxations suggest that relaxations originating from crystalline defects shifts to higher temperatures upon rolling correlating well with the observations that were made using calorimetric transitions.

Keywords: Rolling; poling; β-phase; crystallinity; storage modulus; dielectric permittivity; crystalline/amorphous blends

Stochastic simulations of affinity maturation in germinal centers suggest optimal passive immunization protocols

Amar Garg, Rajat Desikan and Narendra M. Dixit

Passive vaccination using antigen (Ag)-antibody (Ab) complexes to expedite affinity maturation in germinal centers (GCs) and the elicitation of strong humoral responses relies on the choice of Ab employed in the complexes. Any GC B cell attempting to acquire Ag must rupture ICs by its B cell receptor (BCR) binding to the Ag with higher affinity than the complexed Abs, and ''tug" the Ag away. This ability to acquire Ag defines GC B cell fitness. The affinity of the complexed Ab to the Ag relative to the affinity of the BCRs to the Ag thus defines the speed and extent of affinity maturation. Based on this premise, we construct a stochastic evolutionary model of the GC reaction in the presence of passively administered Ag-Ab complexes. Model predictions capture previous experimental observations of reduction in GC volume, increase in B cell apoptosis, and increased rates of affinity maturation with increased affinity of the complexed Ab for Ag. Using this model, we find that an optimum Ag-Ab affinity in the administered complex exists that elicits the best endogenous Ab response. Low affinity complexes fail to drive adequate affinity maturation by not presenting a sufficient selective advantage to higher affinity B cells, whereas very high affinity complexes abrogate Ag acquisition by GC B cells, causing termination of the majority of the GCs. Intermediate affinities strike a balance between enhanced selection pressure to expedite affinity maturation and adequate Ag acquisition by GC B cells to ensure GC survival. The model also shows how this optimum can be tuned by altering Ag availability in the GCs through modulating dosing protocols. Our framework can thus serve to elucidate therapeutic guidelines for vaccine design and Ab-mediated immune regulation.

Funding: Welcome Trust/DBT India Alliance Senior Fellowship

Molecular Dynamics Simulations Reveal The Role Of Membrane Cholesterol During Pore Forming Pathway Of Cytolysin A

Amit Behera and K. Ganapathy Ayappa

Cytolysin A (ClyA), an α pore-forming protein expressed by E. Coli as a water-soluble monomer, undergoes a large conformational change during pore formation. Although cholesterol is known to enhance the lytic activity of ClyA, molecular aspects of the interactions between cholesterol and ClyA during pore formation are not well understood. Using all-atom molecular dynamics simulations ranging from 0.5–0.9μs, we study a single membrane-inserted protomer, a dimer (two protomers) and the dodecameric ClyA pore embedded in a DOPC+30% cholesterol bilayer. In the single membrane-inserted protomer, high cholesterol occupancy was observed around the transmembrane residues of N-terminus which form part of a CRAC motif and also around residues of the β-tongue. Although high cholesterol occupancy sites were not observed near the N-terminus in the dimer simulations, a cholesterol molecule was preferentially located in the pocket formed between two adjacent β-tongues of the dimer. Cholesterol spent 97% of the simulation time (600ns) inside this pocket sampling two major orientations. Energies of two conformations were reported from docking simulations. Formations of transmembrane water channels were observed in both single membrane inserted and dimer ClyA simulations. From the dodecameric pore simulations, density map showed regions of high cholesterol population between the β-tongue pockets and mobility map indicated slower cholesterol in the vicinity of the pore as compared to bulk. Our simulations elucidate specific interactions with cholesterol that could stabilize both the single membrane inserted protomeric state as well as the dodecameric pore.

Water Gas Shift Reaction over Ionic Substituted Manganese Oxide Catalysts

Anil Chilmula and Giridhar Madras

Manganese oxide (Mn_3O_4) and noble metal substituted (Mn_3O_4) catalysts have been synthesised using sonochemical synthesis method. Water gas shift reaction was performed over all the catalysts. It was found that these catalysts are tetragonal structure of (Mn_3O_4) and H_2-TPR analysis showed high oxygen storage capacity. Kinetic parameters were estimated over Pt substituted (Mn_3O_4) catalyst and the reaction was followed redox mechanism. Further catalysts were characterized using XRD for structure, XPS for composition and TEM for morphology and BET for surface area. All the results suggest that Pt substituted (Mn_3O_4) exhibited best activity with maximum stability.

Origami based functional DNA nanotechnology and bio-sensing through DNA aptamers

Banani Chakraborty, Ramalingaswami (DBT) Fellow

DNA origami based nanotechnology and its various applications will be the focus of this talk. First goal is to make bio-nano-chip for multiple targets through DNA aptamer based sensing. Towards that vision, we started off by optimising the binding of multiple aptamers; targeting small molecules and proteins using various fluorescence based techniques such as bulk FRET, PIFE and smFRET. In parallel other set of aptamers are being optimised for sensing particular group of bacteria using gel electrophoresis. All different apia-sensors will be eventually implanted on nano-biochip made of DNA origami. Another area where we are planning to use porous 3-D origami is for cargo delivery vehicle. 6-helix bundle DNA origami of 400nm unit length is already characterised and modified with paramagnetic Iron oxide nanoparticles. Once fully optimised this kind of system will be controlled by external magnetic field which will be programmed with disease targeting aptamer for drug accumulation and release in the affected area. Finally will share the application of DNA 2-D rectangular origami with precise nano pore size for controlling porosity of solid-state nano pore made by Silicon Nitride and Graphene by making hybrid multilayered pores. With molecular dynamic simulation the pore size and pore specificity have already been optimised and the different layers of nano pores are being characterised currently. In summary, we will use the size precision of DNA origami and target specificity of DNA aptamers to take a step towards merging top down and bottom up nanotechnology to make detection of targets in parallel in a practical and cost effective manner.

Imbibition and partial saturation in paper-based microfluidic devices

Dharitri Rath and Bhushan Toley

Paper-based microfluidic devices offer unique advantages over the traditional microfluidic devices having low cost of fabrication, rapid visual signal generation, low reagent consumption and operation with minimal or no ancillary equipment. The imbibition rates in such devices varies with the extent of saturation since the porous structure of the membranes contain pores of multiple sizes having partially saturated regions. Thus the material properties such as the capillary pressure and permeability change as a function of saturation. The phenomenon of partial saturation is not well studied in the paper microfluidics community, and the two most widely used models i.e., the Washburn equation and Darcy's law, to determine the flow rates do not account for the partial saturation at the advancing fluid front. In the current work, we provide a framework for the measurement of imbibition rates as well as the extent of partial saturation as a function of the material properties. A set of experiments were conducted to measure the relationships between capillary pressures, permeability with the extent of saturation for the commercially available paper materials. These experiments can be performed using commonly available lab instruments. For modelling imbibition into paper materials, we solved Richard's equations, taking partial saturation into consideration. Solution of the Richard's equation (in COMSOL) were in strong agreement with the experimental measurements of the imbibition rates as well as the spatiotemporal saturation. Our methods, thus, can be used to precisely determine fluid imbibition and could be used to reduce the trial and error involved in designing paper networks for paper-based microfluidic devices.

Steam Methane Reforming over Cobalt Titanates

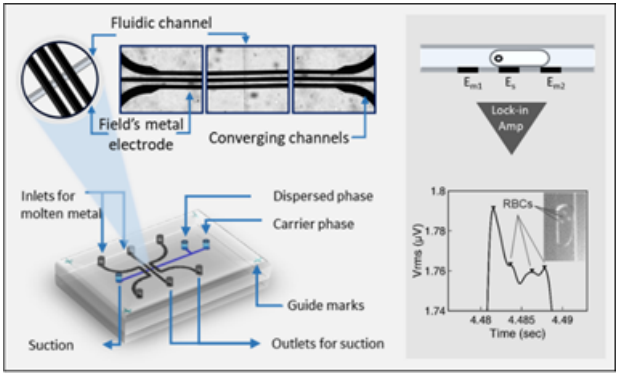
Disha Jain and Giridhar Madras

Steam reforming of methane (SRM) is an extremely important process for the production of ammonia, methanol and other chemicals and in fuel cell applications. Ni-based catalysts have been extensively studied for SRM due to their high catalytic activity and low cost. However, these catalysts suffer with deactivation due to coke deposition and sintering. To overcome the problem of coke deposition, high steam to carbon feed is used, which is not energy efficient. Therefore, the development of coke resistant Ni catalyst is highly desirable. In this study, we have developed a co-doped Ni-Pt catalyst with $CoTiO^3$ as a support pertaining to high thermal stability and oxygen conductivity of the perovskites. The amount of Ni and Pt were optimized to obtain strong metal support interaction. The change in elemental composition was followed by XPS, to examine the involvement of various elements in the reaction. In *situ* FTIR was performed to gain insights into the nature of surface intermediates. The catalytic studies were performed at differential operating conditions and the rate coefficients were obtained using a derived model.

Fusible alloy microelectrodes integrated microfluidic impedance cytometry for cell-in-droplet quantification

Jatin Panwar and Rahul Roy

Microfluidic impedance cytometry (MIC) provides a non-optical and label-free method for single cell analysis1. However, the cleanroom intensive infrastructure required for MIC electrode fabrication limits its implementation. As an alternate approach that enables rapid prototyping, we fabricated Field's metal 'in-contact' (icFM) coplanar microelectrodes in multilayer elastomer devices with a single photolithography step and characterised them for microfluidic impedance cytometry. Our icFM microelectrodes matched performance of the state-of-art platinum electrodes in the detection of single human erythrocytes and water-in-oil droplets in a feedback-controlled suction-flow MIC setup. Finally, to facilitate droplet based single cell analysis2, we demonstrate detection and quantification of single erythrocytes entrapped in water-in-oil droplets.



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Samuels, Proceedings of the National Academy of Sciences 106 (34), 14195 (2009).

Origin of secondary flows in Sheared Granular Materials

Peter Dsouza and P. R. Nott

Recent work by Krishnaraj and Nott [1] has shown the presence of a secondary vortex that forms when dense granular materials are sheared in a cylindrical Couette device. This vortex was shown to explain the presence of a stress anomaly observed by Mehendia et. al. [2]. Krishnaraj and Nott proposed that this vortex was driven by shear-induced dilation.

We show through DEM simulations that the dilation-driven vortices are probably a generic feature of granular flows when the directions of shear and gravity are no co-linear. We show the presence of such vortices in rectilinear and cylindrical "split bottom" Couette cells [3]. We show how the form and extent of the vortex changes as a function of fill height, as a result of changes in the location of the shearing zones. These simulations add to our understanding of how dilatancy of sheared granular materials can explain the form of these vorticies, and indicate the importance of incorporating dilatancy in continuum models for dense granular flows.

References:

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[2] Mehandia, V., Gutam, K.J. and Nott, P.R., "Anomalous stress profile in a sheared granular column," Physical review letters, 109(12), 128002, (2012).

[3] Dijksman JA, van Hecke M. "Granular flows in split-bottom geometries," Soft Matter, 6(13) 2901-7, (2010)

The Structure, Dynamics and Relaxation of Water Confined in Graphene Oxide Slit Pores

M. Rajasekaran and K. Ganapathy Ayappa

Graphene oxide (GO) membranes have attracted a lot of interest due to ease of synthesis, exceptional water permeation properties and precise molecular sieving of ions. As a consequence, graphene oxide membranes may find its application in efficient water purification and desalination technologies. We have used molecular dynamics simulations to study the structure, dynamics and thermodynamics of confined water by constructing a slit pore made up of two surfaces of GO. In order to mimic the different confining situations that occur in GO membranes, we have constructed a GO surface consisting of strips of functionalized regions and studied pores made up of extended GO surfaces, where the functionalized regions are either in-registry (IR), out-of-registry (OR) as well as pores with fully functionalized surfaces (O) and a Janus pore (J) made up of one GO surface and a graphene surface. Inter-surface separations were varied from 0.8 nm to 1.5 nm and we have investigated translational and rotational dynamics, dipole-dipole relaxation as well as the entropy of confined water and compared these across the different GO confining environments. The in-plane diffusion coefficients were higher for the IR pores when compared with the OR pores and in all cases were found to be lower when compared to bulk water even at the largest separations of 1.5 nm. The highest values of diffusion coefficients were observed for the J surfaces. A distinct plateau regime in the mean squared displacements was observed for the smaller pores indicative of glass-like dynamics in these systems. The dipole moment distributions were peaked around 90ofor all cases except for the O surface where two distinct peaks are observed. Relaxation times for the water dipole-dipole correlations were similar for both the IR and OR pores (10 - 300 ps) with distinct differences in the relaxation times only for the 0.8 nm pores. Water in the O and J surfaces showed the slowest and fastest relaxation times respectively. Similar trends were observed for the water translational entropies computed using the two phase thermodynamic model. Interestingly water rotational entropies for the O and J surfaces were found to be slightly higher than that of bulk water. Our study indicates that the structure and ordering of water in GO membranes is a strong function of the

local environment of the water molecules and this study attempts to investigate these different situations by constructing different model systems. Clear signatures of extended and slow relaxation of water at the 0.8 – 1.0 nm pores are signatures of frustrated water dynamics at these separations.

Mathematical model unravels the orchestration of lasting viremic control upon early bNAb therapy in SHIV infection

Rajat Desikan, **Rubesh Raja** and Narendra M. Dixit

The human immunodeficiency virus (HIV) infects the immune system and eventually results in acquired immunodeficiency syndrome (AIDS). To assess therapeutic interventions, macaques infected by simian-human immunodeficiency virus (SHIV) are commonly used as animal models in multiple studies. One such study found that early treatment with a combination of two HIV-1 broadly neutralizing antibodies (bNAbs), 3BNC117 and 10-1074, post SHIV challenge resulted in viremic control in 10 of 13 macaques and arresting their progression to AIDS [1]. They argue that this control, lasting long after the administered bNAbs are cleared from circulation, is due to the effector function of cytotoxic $CD8^+$ T lymphocytes (CTLs). The mechanisms by which early bNAb therapy induces this lasting CTL effector function, however, remained unexplained. To identify the mechanism of control, we constructed a viral dynamics model incorporating bNAb therapy. We confirmed that bNAb therapy indeed increased antigen presentation and CTL stimulation. In addition, by rapidly suppressing viremia early in infection, bNAbs crucially prevented CTL exhaustion. Our model quantitatively captured the measured viral load data only when both these effects of bNAbs were accounted for. We thus argue that it is the combination of enhanced CTL stimulation and reduced CTL exhaustion, the latter from enhanced viral clearance, that underlies the lasting viremic control established by early short-term bNAb therapy.

Reference:

[1] Nishimura, Y. et al. Early antibody therapy can induce long-lasting immunity to SHIV. Nature 543, 559–563 (2017)

Protein-induced fluorescence enhancement as aptamer sensing mechanism for thrombin detection

Saurabh Umrao, Vasundhara Jain, Anusha, Banani Chakraborty and Rahul Roy

Aptamer-based protein sensing can be implemented using a variety of optical and non-optical detection methods. In this report, we present protein-induced fluorescence enhancement (PIFE) based detection of DNA aptamer binding to thrombin. We demonstrate that PIFE reports on direct binding of thrombin to the aptamer strand carrying the fluorophore and hence is unaffected by salt based stabilization of the aptamer conformations as observed in fluorescence resonance energy transfer (FRET) assays. PIFE based thrombin detection displayed excellent linearity in the range of 0.25 pM-25 nM with a detection limit of 8.9 pM. In an alternate scheme, PIFE was demonstrated by placing the fluorophore on a connector strand thus bypassing the requirement to label the aptamer directly. This strategy allowed us to examine thrombin binding by variously modified thrombin aptamers and can serve as a platform for aptamer screening. We finally show that PIFE based thrombin aptamer assay displays high specificity even in the presence of 'natural' background such as blood plasma. We hence propose PIFE based aptamer sensing of protein ligands as a facile yet sensitive and general strategy for biosensing.

A simulation study of Homogeneous Nucleation of molecular solids

Sita Kalyani and Sudeep Punnathanam

The ability of a compound to exist in different crystal structures is called polymorphism and such crystals of varied spatial arrangement are called polymorphs. These polymorphic forms can differ in their properties like dissolution and solubility, chemical and physical stability, bioavailability etc. Due to this, studying polymorphism is important for various pharmaceutical applications. Since crystallization is one of the crucial steps for the separation and purification of drugs, control of the formation of a polymorph depends strongly on the operating conditions of crystallization.

The first step of crystallization is nucleation which involves the formation of a new phase from a supersaturated parent phase. The free energy of formation of a nucleus (ΔG) during nucleation goes through a maximum when plotted against the size of the nucleus (n). The nucleus size at which G is maximum is called the critical nucleus size (n^*) and the free energy of formation of such a cluster is the free energy barrier of nucleation (ΔG^*). Nuclei smaller than the critical nucleus dissolve into the metastable phase and nuclei larger than the critical nucleus grow in size.

In our study, we investigate the phenomenon of homogeneous nucleation of both the polymorphs (Form I and Form II) of a flexible molecular compound – Orcinol ($C_7H_8O_2$). Form I and Form II are obtained experimentally by evaporation of the solvent from Chloroform and Nitromethane solutions respectively. We aim at calculating the free energy barrier (ΔG^*) of both the polymorphs from a supersaturated Orcinol vapor phase as well as from supersaturated solutions of Chloroform and Nitromethane, to identify the most stable polymorph in each case.

Paper Based Non-Enzymatic Glucose Sensor

Sushant Kumar and S Venugopal

Glucose detection has been an area of interest over few decades because of the consequences of low and high sugar level in blood like cardiac, nervous and vascular diseases etc. Today, glucose sensors are readily available and approximately cover 85% of the biosensor market [1]. Electrochemical sensors give an option for economic, low detection limit and enhanced response times for glucose detection in comparison with sensors based on other detection techniques [2]. Most of the commercially available devices are Glucose Oxidase enzyme-based sensors because of high selectivity of enzyme towards glucose. The primary issue is that their sensitivity and performance depend upon the activity of the immobilised enzymes which degrades with time and reading is affected by humidity and temperature. Hence, to eradicate challenges associated with glucose oxidase and reduce glucose strip cost, the scientific community is putting effort to develop fourth-generation enzyme-free glucose sensing technology, also known as a non-enzymatic glucose sensor. Non-enzymatic glucose (NEG) sensors are of various forms depending upon materials used for fabrication. Metals, metal compounds, bimetallic composites/alloys, metal oxides and carbon-based nanostructures are used for fabrication of NEG sensors. Copper being economical and readily available in bulk has gained interest because of its electrochemical activity which makes it suitable for glucose sensing [3]. We report the fabrication of paper-based non-enzymatic glucose electrodes using inkjet printing technology. The base of the electrode is silver nanowire, grown in-situ by a photographic method using inkjet printing and used CuO nanoparticles for detection of glucose. Ag nanowires modified with CuO nanoparticles shows an excellent response to glucose in the range of +0.2V to +0.8V in alkaline medium.

References:

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HIV Evolution in Transmission Potential Landscape

Ananthu James and Narendra M. Dixit

Over the past two centuries, thanks to the advancements in medicine, the lifespan of humans has increased by at least a factor of two. However, still there are many diseases that pose serious threats to human health. One among them, Acquired Immunodeficiency Syndrome (AIDS), caused by Human Immunodeficiency Virus 1 (HIV1), is responsible for around 40 million deaths globally and has no cure currently, though the existing treatments are successful in extending the lifespan of patients by a few years. Recent studies have shown that the set point viral load (the viral density when the symptoms of the disease are absent), which is a main indicator of the disease progression, is optimized so as to maximize viral fitness or transmission potential. Nevertheless, evidences based on comparison between Indian (HIV1-C) and Western (HIV1-B) subtypes suggest that HIV fitness is determined by viral load as well as reproduction ratio (the number of secondary infections by each infected cell), which can vary independently. In this light, with the help of the clinical data corresponding to various subtypes of HIV1, we aim to study how HIV evolves in this 2D fitness landscape towards its optimum.

Kanamycin detection by FRET signal analysis using smart phone based portable device

Anusha, Saurabh Umrao, Vasundhara Jain, Banani Chakraborty and Rahul Roy

Aptamers are RNAs and DNAs originating from in vitro selection experiments (termed SELEX: systematic evolution of ligands by exponential enrichment) which, starting from random sequence libraries, optimize the nucleic acids for high affinity binding to given ligands [1,2]. Aptamers have become increasingly important molecular tools for diagnostics and therapeutics. In particular, aptamer based biosensors possess unprecedented advantages compared to biosensors using natural receptors such as antibodies and enzymes [3]. With the advancement in fluorescence based techniques which gives reduced noise and high sensitivity, it is a great tool for aptamer based bio-sensing. Aptamer target recognition can be monitored by conjugating dyes to it. Here, we report that Förster Resonance Energy Transfer (FRET) between Cy3 and Cy5 dye pairs on the DNA aptamer can enable detection of kanamycin antibiotic. Our detection scheme relies on ligand binding-induced changes in the pre-folded aptamer tertiary structure. We demonstrate the largest detection range for a FRET-based kanamycin binding aptamer (KBA) in a linear range of 0.05-5 nM with a detection limit of 0.18 nM. Further to check the reusability and robustness of KBA, it was surface immobilised and signals of kanamycin binding to aptamer were recovered multiple times. Apart from using a commercial fluoro-spectrophotometer, we in-house built a portable and cost-effective smartphone based fluorescence detector which weighs <120 g and could detect kanamycin in a linear range of 50-500 nM with a limit of detection (LOD) of 28.06 nM using only 10 nM of kanamycin aptamer assay.

References:

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Photocatalytic properties of immobilized $AgBiO_3$ on cellulose acetate membrane for bacteria inactivation and 4-Nitrophenol degradation

Bhanupriya Boruah, Giridhar Madras and Jayant Modak

This study focuses on the synthesis of $AgBiO_3$ nanoparticles by the hydrothermal method. The nanoparticles were immobilized on cellulose acetate to eliminate the separation of nanoparticles. The catalyst was characterized using XRD, XPS, UV-vis DRS, PL, SEM, TEM and BET. ICP-MS analysis was performed to quantify the amount of Ag ions leaching from the powdered and immobilized $AgBiO_3$. The optical and electrochemical properties of the material were analyzed by DRS / UPS and Mott Schottky plot. The band gap of $AgBiO_3$ was estimated by Tauc plots that indicated it is a visible light active photocatalyst. The photocatalytic activity of $AgBiO_3$ was explored for the degradation of 4-nitrophenol and inactivation of E. coli. Kinetic studies and stability tests were performed. Scavenger experiments were carried out to uncover the active species responsible for the photocatalytic mechanism of the reaction and it was found that the superoxide radicals were responsible for the activity.

Uniform rehydration of a sample fluid on dried paper membrane using microfluidic distributor

Debayan and Bhushan Toley

Ever since the development of the first commercially available devices in the 1990's, microfluidic technologies have been evolving to benefit an ever-greater range of applications. Over the last decade, one particularly promising real-world application to emerge has been Point-of-Care (POC) diagnostics. POC tests have the potential to vastly improve health care in a number of ways, ranging from enabling earlier detection of disease, easier monitoring and increased personalization, to reaching under-served and remote populations. In the recent past, one of the growing problem of the developing world is that of the effective diagnosis of various infectious diseases. The testing of such diseases often requires that clinical samples such as sputum, blood, urine, etc. to be transported from the site of collection to the nearest laboratory for analysis. In the course of time, such samples gets spoiled and often they become unfit for analysis when they reach the laboratories. The main motivation of the current work is to build a device that can dry and stabilize large volumes ($>$ 1ml) of liquid clinical samples for downstream analysis. Microfluidic technology is used to develop a distributor that can uniformly distribute sample over a large area of a dried paper membrane. It has been found experimentally that the direct injection of the fluid sample on the dried paper membrane creates a major non-uniformity in distribution and that is highly undesirable. It is expected that the sample collection capacity of paper-based stabilization membranes can be drastically enhanced by using a microfluidic channel network (distributor).

Mass Transport from Walls of Soft Micro Channels

Kaustubh Badwekar and V. Kumaran

The objective of the current study is to quantify and compare mass transport from the soft walls of micro-channels before and after transition to turbulence. PDMS (poly dimethyl siloxane) gel is used to make rectangular channels, three walls of which are hard and one is soft. Vemra & Suran (2016) presented a technique for direct visualization of water transport across nano-membranes. This technique exploits the strong thickness dependent color response of a thin Germanium film ($\sim 25nm$) deposited over a gold substrate. The same property of the Ge nano-film on Gold is deployed here to study mass flux from the wall. Germanium is deposited on the soft wall using sputtering. The etching of Germanium from wall into flowing water in the channels is imaged continuously. The thickness of Germanium is monitored by analysing the colour values (Red, Green and Blue - RGB). Measuring the thickness, otherwise, inside the channels is not possible by the conventional profilometry.

The study of etching was performed on silicon wafers for standardization of the experimental procedure. Also, Ge was deposited on PDMS substrate for the optimized deposition times.

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Printed Electrodes for Polymer Electrolyte Membrane Fuel Cells

Khantesh Agrawal and Venugopal S

Polymer electrolyte (or proton exchange) membrane fuel cells (PEMFC) are an energy-efficient alternative to combustion engines for automotive [1] and are on the cusp of mass-production. Significant advances have been made in PEMFC system design over the last three decades in terms of cost-reduction and structural design. The membrane electrode assembly (MEA), especially the electrode, is considered as 'the heart' of a PEMFC and is designed to accommodate constraints imposed by the cost of platinum used for electrocatalysis, as well as the need for efficient transport of electrons, reactants and heat. Consequently, the structure and composition of the 'electrode' has been significantly altered over the years, from utilizing platinum black films with a platinum loading of 10 gpt/cm^2 in 1970s to present-day platinum/PGM nanoparticle coated carbon black particles (Pt/C) that use about 0.3 mgPt/cm^2. The use of highly-dispersed nanoparticles on carbon black particles enables large gains in surface area for a given mass of catalyst, but concomitant durability problems due to carbon support corrosion and loss of surface area under PEMFC working conditions, especially during start-up or shut down cycles have led to renewed interest in carbon-free nanostructured electrodes, which employ a thin coating of platinum or PGM based catalytic layer on a mesostructured conductive support. In this context, there is scope for novel designs to further reduce platinum/PGM loading of thin film nanostructured electrodes.

The print-expose-develop process developed in our group [2] has been used to fabricate porous, conductive, silver nanostructures on nafion membranes. We are currently working on utilizing a self-terminating process for platinum monolayer deposition [3] onto the silver nanostructures to form conductive, porous, electrocatalytic active catalyst layers for PEMFC applications.

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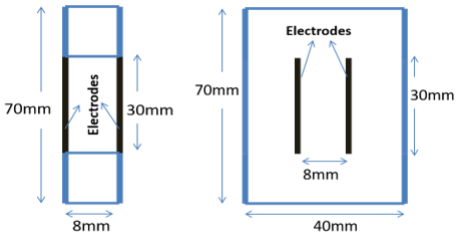
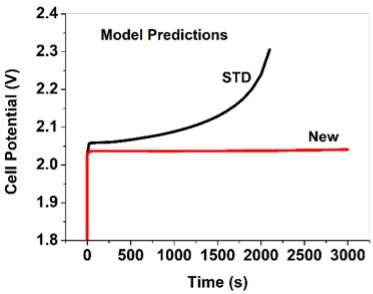
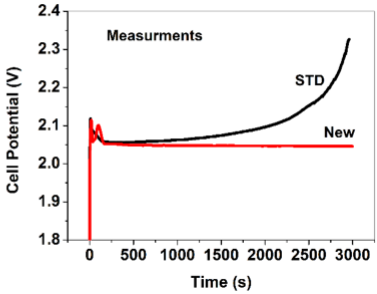
Harnessing natural convection in redox flow batteries: Proof of concept

Md Aslam Ansari and Sanjeev Kumar

Harvesting of renewable energy requires efficient energy storage systems. Rechargeable flow batteries offer certain advantages over other energy storage techniques in use, such as lifting of water, compression of air, flywheel, etc. In a flow battery, electrolyte flowing through/between the two electrodes, electrical energy is stored as chemical energy through reversible redox electrochemical reactions. Typical applications of large scale storage include load-leveling, peak shaving, backup power, electric vehicles, etc. While some flow cells have been scaled up, we still need to develop flow batteries with high energy and power densities, large cycle life, and low cost.







Soluble lead redox flow battery (SLRFB) is among the least expensive in its class, because of the raw material used and the single electrolyte flow loop which eliminates the expensive proton exchange membrane. Challenges such as limited cycle life and low energy efficiency need to be overcome, however, to take it to the next label. In our research group, we have established through CFD-electrochemical reaction modeling, measurements, and flow visualization the dominant role of natural convection in SLRFB. In this work, we present our efforts to harness natural convection for efficient battery designs that need minimal mixing during relaxation between charge-discharge cycles. The figure on the right schematically shows a standard and a new lift cell (electrodes lifted off cell walls). The figure below shows increase in charging potential with time, based on model predictions and measurements. The measurements not only validate the model predictions but also corroborate the effectiveness of the new design—charging continues at a constant low voltage till 3000 s. The improvement brought about with the new design has led to detailed investigations using Particle Image Velocimetry (PIV) for flow validation and testing of other novel designs.

Low-Cost Electromagnetic Valves for Paper-Based Microfluidic Devices

Mithlesh Meena and Bhushan Toley

Paper-based microfluidic devices consist of networks of porous materials capable of moving fluids by wicking. Although paper-based lateral flow assays have been widely used across the globe, few limitations are yet to be completely resolved. In two-dimensional lateral flow assay, flow control is required to do multi-step assays. The primary advantage of paper-based microfluidic devices is that pumps are not required to control the movement of microliter quantity of fluid. Fluid flows in the paper channel by the action of capillary phenomena. In this work, a low-cost electromagnet valve was developed by using screw and copper wire. Wire selection for electromagnet depends on the screw and wire geometry, as well as the electric property of wire defined by experimental observations. The developed ON/OFF switch valve operates at 2.1Volt DC supply and observed current is 4. 64Ampere.The distance between two channels is less than 1mm. Two types of setup for channels which are parallel and perpendicular demonstrated with the position of electromagnet valve.these setups are useful in desire purpose of channel arrangement in the device. Our major focus of interest is to control the fluid flow, hold the fluid in channels, and deliver fluid from one channel to another, using this valve. These paper-based microfluidic devices are beneficial in disease diagnostics in both developing and developed countries. They are rapid, inexpensive, portable, easy to use, and do not require skilled workers to operate.

Highly Sensitive Point-of-Care Immunoassays based on paper microfluidics

N Sathish and Bhushan Toley

An important biomarker for which an ultra-sensitive POC immunoassay must be urgently developed is Lipoarabinomannan (LAM), a urine biomarker of active tuberculosis (TB) infection [1,2]. Several field trials have been conducted with two commercially available LAM tests based on immunoassays – Clearview TB-ELISA and Determine TB-LAM test (Alere; Waltham, MA). The Determine TB-LAM is a lateral flow RDT compatible with POC testing. Although the clinical sensitivity of these tests for the detection of TB has been low for non-HIV infected patients (6%-21%) [3], moderate clinical sensitivities (67%) with high specificities (>96%) were obtained in HIV TB co-infected patients with CD4 cell counts $<100/\mu$l [2,4]. This latter group represents a population in which TB is especially difficult to diagnose. Urine LAM testing can reduce the mean time to diagnosis in this group by $\sim$3 weeks [4] and enable earlier onset of treatment. Detection of LAM can also be used to monitor TB treatment [5,6]. The limited clinical sensitivity of current LAM immunoassays is at least partly because of the inability of these tests to detect LAM at sufficiently low concentrations. In a laboratory study, when urine was concentrated 100x, the clinical sensitivity of the LAM immunoassay improved from 7% to 57%. Recent article showed that the LAM can be detected with high sensitivity for non-HIV infected patients [7]. This shows clearly that there is need for more sensitive POC immunoassays than current tests. In this project, an ultra-sensitive immunoassay for the detection of urinary LAM will be developed by using various signal enhancement strategies. The different fluidic and chemical operations involved in this assay will be automated in paper microfluidic channels using novel low-cost electromagnetic valves. The devices developed in this project will serve as platforms for conducting ultra-sensitive immunoassays for other biomarkers.

We have developed an enzymatic detection system (HRP-DAB) and Gold nanoparticle-based detection system for the malarial biomarker (PfHRP2) as a Model analyte. The process of implementing the same

strategy for LAM as analyte for detection of TB is on-going.

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Paper-based microfluidics for rapid and low-cost DNA testing

Navjot Kaur and Bhushan Toley

The genetic material of any organism is its unique identifier and nucleic acid amplification techniques (NAATs) exploit this very principle for the detection of various biological species. NAATs are extremely specific and rapid, with the ability to detect up to a few copies of the pathogen and detect mutated pathogen strains. These characteristics make NAATs highly compatible for point-of-care (POC) diagnostics, to potentially fulfil the ASSURED (Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment free and Deliverable to end users) criteria for POC devices as established by the World Health Organization (WHO). Currently, the polymerase chain reaction (PCR) is the gold standard for NAATs, widely used in clinical laboratories. But there exist limitations of exorbitant costs of the PCR machine and requirement of a trained personnel to carry out the reactions, making it incompatible for POC diagnosis. The goal of my research is to develop a POC diagnostic device for Tuberculosis diagnosis. The device would incorporate an isothermal NAAT named Loop mediated isothermal amplification (LAMP) and paper-based microfluidics. Two LAMP assays, targeting two different genes of Mycobacterium tuberculosis (MTB) have been standardised in the lab and the assays have been successfully ported into paper substrates. The assay will now be combined with a lateral flow detection technique and built into an integrated device. Further efforts will then concentrate at dry storage of reaction reagents within the paper matrices, incorporating multiplexing capabilities, detection of mutations in MTB correlated to Rifampicin resistance and sample preparation; to enable the vision of a sample-in-answer-out device starting from sputum samples.

Non-catalytic synthesis of fatty acid methyl esters (FAMEs) using supercritical fluids

Neha Lamba, Giridhar Madras and Jayant Modak

Fatty acid methyl esters (FAMEs) are useful as biodiesel and have environmental benefits compared to the conventional diesel. These esters can be synthesized with or without catalyst. Conventionally, acid or base catalysts are used to synthesize these esters. The catalytic route of synthesis requires high production cost because of the pretreatment of feedstock for removal of free fatty acids (FFA) and water and for the purification of the product mixture. Supercritical fluid technologies (fluids which are above their critical point and have intermediate properties to gases and liquids) for FAMEs synthesis obviate the need of a catalyst and precludes the pretreatment steps for FFA and water. These fluids have good solvating power which can be tuned by changing both the pressure and temperature. Biodiesel or FAMEs is primarily synthesized using edible vegetable oils and methanol with a catalyst. However, the frequent use of methanol as methylating agent for transesterification has resulted to excess of glycerol in the market. Thus, alternate methylating agents are required.

In this context, FAMEs were synthesized from a non-edible oil (*Calophyllum inophyllum*, sura honne) in different supercritical fluids: methanol (MeOH), methyl tert-butyl ether (MTBE), methyl acetate (MeOAc) and dimethyl carbonate (DMC) non-catalytically. A comparative kinetic study of the reaction of sura honne oil with different methylating agents was performed to understand their potential as substitute methylating agents. Reactions were performed from 523 K to 673 K at 30 MPa with a molar ratio of 40:1 with times varying from 3 min to 3 h. Conversions higher than 80% were obtained within 30 min for oil reaction with MeOH and DMC at 623 K and conversions of 60% and 70% were obtained at 673 K with MeOAc and MTBE, respectively. Pseudo first order kinetics was used to obtain the rate constants and the activation energies followed the order: $E_{MeOH} < E_{DMC} < E_{MeOAc} < E_{MTBE}$.

Lattice Boltzmann Simulation of Nanoparticles in a Lamellar Phase

Poornima Ramamohan and V. Kumaran

Nanoparticles have been shown to have a stabilising effect on emulsions such as oil-water systems under certain conditions by the reduction of the free energy of the system. The interplay of various factors such as hydrophilicity/hydrophobicity of the nanoparticles, nanoparticle size or concentration, ratio of fluid components, etc. have been found to influence phase transitions in multi-component systems and the presence of defect structures in these phases. Oil-water emulsions can also be used for bottom-up formulations of functionalised nanoparticle structures, which in turn can have applications in diverse areas of science and technology such as in the pharmaceutical and cosmetic industries, display technology and information storage, and can also give insight into various biological systems such as the transport across bilpid layers (such as cell membranes). The mesoscopic Lattice Boltzmann method has been employed to simulate the oil-water system using the velocity and concentration fields to characterise the system in two dimensions. Model-H equations, obtained by coupling the Cahn-Hilliard equation for the interfacial evolution and the Navier Stokes equation for momentum and mass transport, have been used to describe the lamellar phase. The nanoparticle(s) immersed in the lamellar phase follow Newtonian dynamics, with the net force on the particle exerted by the fluid being a combination of hydrodynamic forces and hydrophilic/hydrophobic interactions. The first component of force is determined by appropriate solid-fluid boundary conditions, while the second component involves the introduction of a particle-fluid potential that affects the equilibrium velocity of the modelled system. Validation of the LB model has been done by obtaining the equilibrium structure of the lamellar phase, as well as by the visualisation of the fluid's velocity field in the vicinity of the particle.

Modelling synergy between anti-HIV drugs

Pramita Sen and Narendra M. Dixit

The standard HIV treatment regimen comprises a combination of three antiretrovirals, namely, two nucleoside reverse transcriptase inhibitors (NRTI) and a non-nucleoside reverse transcriptase inhibitor (NNRTI). The best triple drug combinations may entail synergy between components. However, a comprehensive understanding of the origin of synergy in drug combinations is missing and people rely on experiments for identifying synergistic drug combinations from an enormous pool of forty antriretrovirals. Here we develop a mechanistic model to explain interaction between antiretrovirals, namely, among NRTIs and between NRTIs and NNRTIs and identify the origin of synergy, if any, in dual and triple drug combinations. We construct a detailed reaction network-based model of the HIV lifecycle, with focus on the stages that are affected by the above drugs. We elucidate the origins of synergy and devise strategies to maximize the synergy. Predictions for the optimal use of these drugs emerge. The model can be applied to identify the best three drug combinations.

Serially diluted droplet generation for nucleic acid quantification

Prithiv Natarajan, Jatin Panwar, Priyanka Valloly and Rahul Roy

Efforts in the direction of integrating nucleic acid quantification (NAQ) within microfluidic or Lab-on-Chip platforms have been promising. Despite the incorporation of 'on-chip' quantification of DNA/RNA copy number (for point-of-care and diagnostic purposes) these quantification methods demand 'off-chip' sample preparations that require serial dilution — a cumbersome manual procedure — which allows a significant margin for erroneous measurements, especially when dealing with volumes of a few microns.

Although on-chip preparations of serially diluted samples using valves or, in some cases, assisted by electronics, are currently available, nevertheless, they do not show the discrete nature of compartments that localise the concentration and are moreover limited in their capacity as high throughput modular devices. Through our research, we describe a modular Microfluidic device integrated with 'on-chip' serial dilution through valve mediated flow control ensuring high dynamic range of dilution, followed by droplet generation for discretisation. This poses increasing relevance in applications involving end-point/real-time isothermal NAQ, barcoding of droplets for 'off-chip' usage, viral load quantification for prognosis and drug response monitoring.

Isothermal Droplet Digital Quantification of Nucleic Acids

Priyanka Valloly and Rahul Roy

Development of tools for fast, specific, sensitive and quantitative detection of virus infections is a critical requirement for diagnosis and disease management. For clinical diagnostics, the gold standard for viral load quantification remains the nucleic acid amplification (NAA) and detection based 'quantitative Polymerase Chain Reaction' (qPCR) [1]. A new alternate to qPCR is 'Droplet digital PCR' which provides an absolute quantification of nucleic acids without requirement for standard concentration calibrations and reference controls [2]. Despite its several advantages, it still requires thermal cycling and slow in detection since amplification needs to be from single molecules. Additionally, it requires multiple devices i.e. for droplet generation, nucleic acid amplification and finally signal detection using imaging. Incorporation of isothermal amplification assays in droplet-based platforms can provide the flexibility of operations with the possibility of single device operation with reduced sample volumes and contamination [3]. Here, we report development of an integrated digital droplet NAA microfluidic device based on isothermal amplification technique for absolute viral load quantification. This digital microfluidic droplet generator is an integrated platform for carrying out all the unit operations needed for the study, like capturing of nucleic acids, uniform distribution of reagents and samples, mixing of reagents, amplification and detection of nucleic acids. Highly monodisperse droplets are generated by encapsulating viral nucleic acids, amplification reagents and a fluorescent marker. These act as micro reactor for isothermal nucleic acid amplification. Target viral nucleic acids are diluted to capture at most single copy per droplet and are amplified and later detected by fluorescence. The "on" and "off" signal (due to presence or absence of viral RNA) from the droplets estimate the bulk concentration based on Poisson statistics. As a first proof-of-principle implementation, we have established Recombinase Polymerase Amplification (RPA), an isothermal (37 - 40°C) amplification method to detect and quantify dengue plasmids and RNA in the droplets within 10 - 12 mins.

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Investigating oligomerization pathways of ClyA pore forming toxin

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Pore-forming toxins (PFTs) expressed by pathogenic bacteria form nanoscale pores on target cell membranes resulting in cell lysis and death [1]. Water soluble monomers of ClyA, a representative α-helical PFTs undergo structural rearrangements to a protomer form upon binding to bilayer membrane and further oligomerize to form stable pores [2]. The molecular mechanisms giving rise to selective pore formation on eukaryotic membranes have been unclear, especially in the case of α-PFTs [3]. Here, we use single particle fluorescence microscopy to examine the events in the pore formation pathway on supported bilayer membranes. We demonstrate how cholesterol is a critical component for effective pore formation and a possible means for selective targeting by PFTs. Binding of ClyA, a representative αPFT from E. *coli* on supported lipid bilayers was rapid and complete within seconds. From diffusional and trajectory analysis of single particles, we observe that the monomeric protein transitions between a high and low mobility state via an intermediate. We argue that the lowest mobility ClyA conformation represents a stable membrane bound intermediate along the assembly pathway. This 'immobilization' kinetic is accentuated in bilayers with cholesterol highlighting its role in ClyA pore formation. Additionally, cholesterol binding at the protomer-protomer interface may be another factor in the enhanced oligomerization process. Dye photobleaching and brightness analysis of ClyA oligomers at high concentrations suggests that cholesterol enhances the fraction of oligomers leading to pore formation possibly by stabilizing the assembly intermediates mainly protomer-protomer interactions. This role of cholesterol at two different time points in the pore-formation pathway of ClyA might explain its molecular mechanism of selectively targeting mammalian cells. Additionally, mutational studies in the regions which are possibly interacting with the cholesterol offer more evidence of ClyA's interaction with the lipid bilayer and will pave a path for modelling the mechanism of pore formation by ClyA. **References:**

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A Robust Thermodynamic Theory for Gas Hydrates

Shivanand Kumar Veesam and Sudeep Punnathanam

Clathrate hydrates are nonstoichiometric crystalline inclusion compounds in which the host lattice is made up of water molecules that are tetrahedrally bonded to each other via hydrogen bonds and guest molecules consist of typically small gas molecules, such as methane, ethane etc [1]. The crystalline structure of the host lattice contain cavity like structures, which are occupied by the guest molecules. Traditionally, thermodynamics of gas hydrates is described by van der Waals and Platteeuw (vdWP) theory [2] which models the clathrate hydrate as an adsorbent and the cavities as adsorption sites. In this theory [2], the host lattice is assumed to be rigid; each cavity can contain at most one guest molecule; guest-water interaction is limited to the nearest neighbor water molecules forming the cavity; and guest molecules do not interact with each other. The guest-water potential parameters and empty hydrate properties are usually regressed from experimental phase equilibrium data [1]. Chialvo et al. suggested that the success of the vdWP theory is due to presence of large number of adjustable parameters used in the regression of equilibrium data and the theory acts as a data correlator. There has been a lot of work over the years to overcome these shortcomings of the original vdWP theory. Several studies showed that guest-water interactions beyond the first cage are significant enough to influence the phase equilibrium predictions. Other studies have pointed out that guest-guest interactions are also significant to influence the phase equilibrium predictions. In this context, Punnathanam and co-workers [3,4] showed that the rigid host lattice approximation is a significant source of error while predicting the phase equilibrium. To overcome this drawback, Ravipati and Punnathanam [5,6] developed a method to compute the contribution of movement of water molecules in the host lattice to the partition function of the clathrate hydrate. The work of Ravipati and Punnathanam [6] successfully demonstrated the accuracy of the modified vdWP theory using the phase equilibrium data computed using molecular simulations. In this work, we apply the modified vdWP theory to model the experimental phase equilibrium data, and recompute the guest-water potentials. The empty hydrate reference properties are directly computed from molecular simulations. The modified vdWP theory al-

lows us to substantially reduce the number of regressed parameters to two per guest molecule while achieving accuracies comparable to those predicted by CSMGem [1] program which contains 13 regression parameters. In addition, our method can also predict the hydrate cage occupancy accurately.

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Modelling how reversal of immune exhaustion elicits cure of chronic hepatitis C after the end of treatment with direct-acting antiviral agents

Subhasish Baral, Rahul Roy and Narendra M. Dixit

A fraction of chronic hepatitis C patients treated with direct-acting antivirals (DAAs) achieved sustained virological responses (SVR), or cure, despite having detectable viremia at the end of treatment (EOT). This observation, termed EOT^+/SVR, remains puzzling and precludes rational optimization of treatment durations. One hypothesis to explain EOT^+/SVR, the immunologic hypothesis, argues that the viral decline induced by DAAs during treatment reverses the exhaustion of cytotoxic T lymphocytes (CTLs), which then clear the infection after treatment. Whether the hypothesis is consistent with data of viral load changes in patients who experienced EOT^+/SVR is unknown. Here, we constructed a mathematical model of viral kinetics incorporating the immunologic hypothesis and compared its predictions with patient data. We found the predictions to be in quantitative agreement with patient data. Using the model, we unraveled an underlying bistability that gives rise to EOT^+/SVR and presents a new avenue to optimize treatment durations. Infected cells trigger both activation and exhaustion of CTLs. CTLs in turn kill infected cells. Due to these competing interactions, two stable steady states, chronic infection and viral clearance, emerge, separated by an unstable steady state with intermediate viremia. When treatment during chronic infection drives viremia sufficiently below the unstable state, spontaneous viral clearance results post-treatment, marking EOT^+/SVR. The duration to achieve this desired reduction in viremia defines the minimum treatment duration required for ensuring SVR, which our model can quantify. Estimating parameters defining the CTL response of individuals to HCV infection would enable the application of our model to personalize treatment durations.

Antifreeze Proteins – A Molecular Dynamics Study

Surbhi Kumari and Sudeep Punnathanam

Antifreeze Proteins (AFPs) have been identified in various organisms inhabiting cold environments. These proteins help in their survival at sub-zero temperatures by binding to the ice-water interface and creating a local depression in the freezing point of ice, due to the Gibbs-Thomson effect. In this study, we have carried out molecular dynamics (MD) simulations to gain an insight of this phenomenon at the molecular level. The retardation of ice growth in the presence of a Type-III AFP at the ice-water interface at various sub-freezing temperatures has been observed. It has been found that the presence of AFP on the interface prevents ice growth even up to 8 degrees of supercooling. Visual study of the system confirms the formation of convex ice fronts due to the binding of the protein to the ice-water interface. An effort has been made to understand the binding mechanism by estimating interaction energies using escape simulations and Potential of Mean Force (PMF) calculations. The PMF profile has been obtained by carrying out umbrella sampling simulations. Thus, due to their antifreeze effect, Type-III AFPs can be potentially used in various industries, especially in the transportation and storage of frozen food items.

Droplet-in-Drop Structure in Agitated Dispersions

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Several rate processes such as direct contact heat transfer, solvent extraction, suspension polymerization, multiphase reactions etc. benefit from high contact area between two liquids, till the rate processes reach completion, followed by complete separation of phases. Agitated dispersions serve the needs of such processes ideally as phase separation is affected readily through density difference between the phases, after energy input to agitator is stopped. Phase Inversion is a common phenomenon that occurs in such agitated dispersions. The mechanism of drop coalescence and breakup directly affects phase inversion, which has been modeled using a Monte-Carlo simulation in this study. One of the descriptions of phase inversion is lack of a stable drop size distribution after increasing the holdup of dispersed phase above a certain value. Droplet-in-drop structure of dispersions is proposed by some authors based on experimental studies and we have attempted to see if it can be incorporated in a Monte-Carlo simulation of the system as well.

Translation to replication switching by resource segregation during Flavivirus life cycle

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Single-stranded RNA (ssRNA) virus infection cycle progresses by resource (viral RNA) allocation to macromolecular complexes: host ribosomes (translation), viral RNA polymerase (replication) and viral structural proteins (packaging). Many positive-sense ssRNA viruses, from *Flaviviridae* family further compartmentalize replication process in membranous vesicles, which is proposed to help in selective resource allocation to viral RNA polymerase. We hypothesized that spatial segregation of replication imparts replicative advantage for the virus by optimizing viral protein to nucleic acid ratio. Using Japanese encephalitis virus infection in neuro2a cells, we measured viral RNA dynamics in the context of fraction of viral RNA undergoing translation, replication and packaging during virus life cycle. We observed high levels of viral translation during early stages of infection as expected for a positive-sense ssRNA virus. However, as infection progressed, viral RNA transitions from a translation dominant phase to a replication phase while the total viral RNA continued to increase. Currently, detailed and quantitative understanding of this transition from translation to replication and packaging phases is missing. We mathematically modelled the kinetics of resource distribution in a well-mixed versus a compartmentalized cell infection model and compared this to Flavivirus infection in cell culture. Our model predicts that this translation to replication switch is inherent to the lifecycle architecture with replication compartmentalization. Perturbation of the modeled kinetic parameters demonstrates drastic changes in viral burst sizes beyond critical switching times, observed in experiments, suggesting multiple steady states for the virus lifecycle. We posit that analysis of intracellular viral dynamics using a combination of experimental and modeling efforts can provide novel insights into viral life cycle.