AUSTRALIAN SOCIETY FOR BIOPHYSICS

44th Meeting of Australian Society for Biophysics

Programme and abstract booklet

All sessions will be conducted via zoom. Links are embedded in the schedule and listed in the table below:

	Stream 1	Stream 2
Wednesday	ASB 2020 – Stream 1 - Wednesday	ASB 2020 – Stream 2 - Wednesday
Thursday	ASB 2020 – Stream 1 - Thursday	ASB 2020 – Stream 2 - Thursday
Friday	ASB 2020 – Stream 1 - Friday	ASB 2020 – Stream 2 - Friday

For single stream sessions, please use the link for Stream 1.

Passcode for all sessions is: 130575

Day 1: Wednesday 2nd December

Morning session

	ASB/UC Davies Biophysics Chapter ECR symposium **Chairs: Dr Amanda Buyan, Dr Katelyn Jarvis** **Zoom:** ASB 2020 – Stream 1 - Wednesday
9.00	Xiawei Zhang. UC-Davis Effects Of Ultrastructural Remodeling On Calcium Signaling And Electrophysiology In A Three-Dimensional Model Of The Human Atrial Myocyte
9.15	Lanie Ruiz-Perez. Curtin University Molecular simulations and enhanced sampling for the permeation of short peptides across a model of the skin barrier
9:30	Clifford TeBay. VCCRI Comprehensive preclinical cardiac safety evaluation of potential COVID-19 drugs
9:45	Katelyn Jarvis. UC-Davis Models of muscle contraction must include force-dependent weakly-bound cross-bridges and series elasticity to describe stretch-activation in *Dropsophila* jump muscle fibers
10:00	Shiying Zhu. Bio21 Institute, University of Melbourne In-cell Structure Determination of an Antimicrobial Peptide by DNP solid-state NMR
10:15	Elsamouny Nehad. University of Wollongong Structural basis for the human/mouse species selectivity of 6-substituted 5-(hexamethylene)amiloride (HMA) analogs as inhibitors of the urokinase plasminogen activator
10:30	Islam Mohammed Zohurul. UTS Understanding the concentration-dependent Interaction of Sterol Drugs with Model Lung Surfactant Monolayers Using Molecular Dynamics Simulations
10:45	Jyoti Gurung. UNSW Sydney Separation and enrichment of sodium-motile bacteria using cost-effective microfluidics

12.50 Opening of the meeting - Adam Hill (President, Australian Society for Biophysics) - ASB 2020 – Stream 1 - Wednesday

Lunch Session

	Session 1a: Ion Channels and Pumps **Chair: Dr Adam Hill** **Zoom:** ASB 2020 – Stream 1 - Wednesday	**Session 1b: Computational Biophysics** **Chairs: Dr Evelyne Deplazes/Dr Andrew Battle** **Zoom:** ASB 2020 – Stream 2 - Wednesday
13.00	Dr Chai Ng. VCCRI Saturation mutagenesis study of heterozygous hERG channel variants using SyncroPatch 384	Jared Collette. University of Melbourne. Membrane Tension Can Enhance Adaptation to Maintain Polarity of Migrating Cells
13.15	Prof Jamie Vandenberg. VCCRI What is the origin of the upside down kinetics of HERG K+ channels	Dr Karen Corbett. Monash. Conformational Determinants of Cyclic Peptide Permeability
13.30	Dr Delfine Cheng. VCCRI Piezo1 channels as regulators of integrin-mediated focal adhesions in cardiac fibroblasts	Hugo MacDermott-Opeskin. ANU. Host-derived lipids impact the membrane dynamics, antibiotic resistance evolution and efflux systems of Acinetobacter baumannii
13.45	Joonhyung Bae. UNSW. Screening for novel sodium motility inhibitor.	Dr Nicholas Smith. Latrobe. Bak Core Domain Dimers Alter Membrane Dynamics; Assessed by Atomistic and Coarse-Grain Simulations
14.00	Viaksh Shah. University of Otago The Role of Epithelial Sodium Channel (ENaC) and Endothelial Glycocalyx in Influencing Mechanical Properties of Endothelial Cells.	Sheikh Imamul Hossein. UTS. Shape Effects of Gold Nanoparticles on Model Lung Surfactant Monolayer
14.15	Nazanin Mohebali. University of Melbourne Purification and structural characterization of TACAN, a novel ion channel implicated in pain sensing	Dr Evelyne Deplazes. UTS. An integrative approach to understand the transport of ions across phospholipid bilayers
14.30	Dr Carus Lau. VCCRI Understanding the structural basis of inactivation mechanism of the hERG potassium channel	Safura Jokar. Shiraz University of Technology. Evaluation of the Intermolecular Interactions between the Peptide-based Inhibitors and Amyloid-β Monomer/Fibril Structures

Afternoon Session

	Session 2: Careers for science graduates **Moderator: Prof Jamie Vandenberg** **Zoom:** ASB 2020 – Stream 1 - Wednesday
15.15	Dr Johanna Barclay. Senior manager. Victor Chang Innovation Centre A 'choose your own adventure' career, on and off the bench
15.35	Dr Romaric Bouveret. Business Strategy manager, UNSW Having an impact beyond traditional career paths
15.55	Dr Emily Finch. Policy Analyst, Australian Academy of Technology and Engineering (ATSE). From research to science policy and back again.
16.15	Melissa Erce, PhD. Patent Attorney FB Rice
16.35	Prof Boris Martinac, VCCRI. A career in academic science
16.55 – 17.30	Panel discussion/Q&A

Day 2: Thursday 3rd December

Morning session

	Session 3: Australian Society for Biophysics/Biophysical Society of Japan joint session **Chairs: Dr Charles Cox/ Dr Shinya Sato** **Zoom:** ASB 2020 – Stream 1 - Thursday
10.00	Dr Shinya Sato. Kytoto University. Detect with PKAchu: Light-off-induced PKA activation in rod photoreceptor cells
10.20	Dr Charles Cox. VCCRI Modified N-linked glycosylation status predicts trafficking defective human Piezo1 channel mutations
10.40	Dr Kohei Otomo. National Institute of Natural Sciences. Improving two-photon excitation microscopy for deeper, sharper and/or faster intravital imaging
11.00	Dr Emilie Flood. RMIT University. Investigation of the gating, inactivation and blockage of a calcium gated ion channels using molecular dynamics simulations and cryo-EM
11.20	Dr Kota Katayama. Nagoya Institute of Technology. Vibrational spectroscopic study of G protein-coupled receptor
11.40	Xuguang Jiang. University of Tokyo. Discovery of an unusual activation mechanism for the TipA multidrug-resistance transcriptional regulator
12.00	Es Darley. UNSW. Interactions of cholesterol-tagged DNA and lipid bilayer membranes

Lunch session

	Session 4: Australian Society for Biophysics NZ section **Chairs: Dr David Baddeley/Dr Matthew Perry** **Zoom:** ASB 2020 – Stream 1 - Thursday
13.00	Dr Juliette Cheyne. University of Auckland *In vivo* calcium imaging in mouse models of disease
13.20	Dr David Baddeley. University of Auckland. High-content super-resolution microscopy
13.50	Michelle Munro. University of Otago. The role of cardiac ryanodine receptor clusters in calcium leak and arrhythmia
14.10	Dr David Crossman. University of Auckland. Fibrosis and impaired Ca^{2+} signalling in heart failure

14.30	Martin Fronius. University of Otago. N-glycans – the sticky ends for shear force activation of epithelial Na+ channel (ENaC)
14.50	A/Prof Tony Hickey. University of Auckland. Mitochondria and the physics of insect flight

Afternoon session

	Session 5: Computational biophysics **Chairs: A/Prof Adelle Coster/TBD** **Zoom:** ASB 2020 – Stream 1 - Thursday
15.30	Dr Stewart Heitmann. VCCRI. Initiation of Cardiac Arrhythmias by Heart Cells that Fail to Repolarise
15.45	Hossein Borazjani. Shiraz University of Technology. The potential role of the physical and chemical arrangement of erythrocytes membrane molecules on its mechanical behavior: A molecular dynamics simulation
16.00	Dr Omid Bavi. Shiraz University of Technology. Theoretical studies on the selectivity of inhibitors on FGFRs by molecular dynamic simulations and free energy calculations
16.15	Sandra Moore. Curtin University. The Influence of the Choice of Force Field on the Characterisation of the Monomeric Form of Rat and Human Islet Amyloid Polypeptide
16.30	Krushna Sonar. Curtin University. Characterization of amyloid β peptide structural ensemble
16.45	Zahra Nickfarjam. Shiraz University of Technology. A Molecular Docking and Molecular Dynamics Study on Prostate-Specific Membrane Antigen (PSMA) to design effective small molecules as inhibitors
17.00	Dr Vijay Rajagopol. University of Melbourne. How does calcium make the heart grow?

17.30 – Australian Society for Biophysics Annual General Meeting

Zoom : ASB 2020 – Stream 1 - Thursday

Day 3: Friday 4th December

Morning session

	Session 6: Plenary/prize session. **Chairs: Prof Boris Martinac/Dr Amanda Buyan** **Zoom:** ASB 2020 – Stream 1 - Friday
9.00	Prof Eduardo Perozo. University of Chicago Force from lipids gating in mechanosensitive channels.
9.50	Dr Sara Pandidan. Latrobe. Locating antimicrobial peptides in model membranes: comparison of the action of carpet and pore forming peptides via neutron reflectometry
10.10	Dr Katie Wilson. ANU. Understanding the biophysical properties of the plasma membrane through computational modelling
10.30	ASB/Davis Prize
10.45	ASB Davis Prize

Lunch session

	Session 7a: General Biophysics **Chairs: Dr Charles Cranfield** **Zoom:** ASB 2020 – Stream 1 - Friday	**Session 7b: Membranes and membrane proteins** **Chair: Prof Frances Separovic** **Zoom**: ASB 2020 – Stream 2 - Friday
12.30	Dr Valentin Romanov. VCCRI. An acoustic platform for single-cell, high-throughput measurements of the viscoelastic properties of cells	Dr Tony Ngo. Structural basis of CXCR4 signalling illuminated by experimental and computational approaches
12.50	Dr Navid Bavi. University of Chicago. Probing mechanosensitivity at the cell-substrate interface	Quilin Yu. University of Melbourne. Positive Feedback Loop Between E-cadherin and F-actin
13.10	Dr Steffi Cheung Tung Shing. University of Melbourne. Deciphering the assembly and signalling mechanisms of the unique	Vijay Kompella. Curtin University. Characterization of slow and sub-diffusive behaviour in crowded protein solutions and discerning the underlying causal relations

	BAFF 60-mer through the TACI cytokine receptor	
13.30	Dr Rocio Finol-Urdaneta. University of Wollongong. Ionic Basis of human Neurogenin-2 induced sensory neuron excitability	Conall McGuinness. UNSW. Perfringolysin O pore formation dynamics: from soluble monomer to membrane insertion
13.50	Dr Jeffrey McArthur. University of Wollongong. Repurposing the spider toxin Pn3a as a selective inhibitor within the T-type calcium channels	Dr Sam Henderson. University of Adelaide. Reconstitution of recombinant human AQP1 into lipid vesicles enables single-channel recordings of AQP1 cation channel activity
14.10	Dr Sami Al-Izzi. UNSW. Asters, spirals and vortices revisited: ATP dependent structures in actomyosin	Bronte Johnstone. University of Melbourne. Formation of a novel two-component pore complex by a homologous pair of pore-forming toxins from Elizabethkingia anophelis

Afternoon session

	Session 8: Viral Biophysics/McAuley Hope **Dr Matthew Baker** **Zoom:** ASB 2020 – Stream 1 - Friday
15.00	Dr Theresa Klemm. WEHI. Mechanism and inhibition of the papain-like protease, PLpro, of SARS-CoV-2
15.20	Dr Christian Morkel. VCCRI. TBD
15.40	Dr Romain Rouet. Garvan Institute. Re-engineering of SARS-CoV-1 monoclonal antibodies for SARS-CoV-2 neutralization
16.00	Josiah Bones. ANU. Towards understanding strain variances in coronavirus pathogenicity by simulating S Protein RBD energetics.
16.20	McAuley-Hope Prize for original biophysics: Dr Till Boecking

17.00 Conference close – Dr Matthew Baker

Day 1: Wednesday 2nd December

ASB/UC Davies Biophysics Chapter ECR symposium
09:00-11:00

Effects Of Ultrastructural Remodeling On Calcium Signaling And Electrophysiology In A Three-Dimensional Model Of The Human Atrial Myocyte

Xianwei Zhang[a], Haibo Ni[a], Stefano Morotti[a], Daisuke Sato[a], William E. Louch[b], Andrew G. Edwards[a], Eleonora Grandi[a]

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Intracellular calcium mediates the bidirectional coupling between excitation and contraction (ECC) in cardiac myocytes, whereby calcium affects electrophysiology and vice versa. Abnormalities in ECC can precipitate arrhythmia in diseased conditions such as atrial fibrillation (AF) and heart failure. In these settings, the altered coupling between calcium signaling and electrophysiology is mediated by both ionic remodeling (i.e., changes in expression and function of channels and transporters) and ultrastructural remodeling (i.e., decreased density and regularity of the transverse-axial tubule system). Ultrastructural remodeling, and particularly tubules, are much less studied than ionic remodeling, in part due to technical challenges. While tubules were considered unique to ventricular myocytes, recent studies have demonstrated the presence of atrial tubules and characterized tubular features in atrial myocytes across several species, including human. To understand the role of atrial tubules in atrial physiology and of their disruption in AF, we built a 3D human-specific atrial model coupling electrophysiology and subcellular calcium signaling with a spatially-detailed description of tubular structure. We generated a population of models with random tubular patterns based on experimentally characterized feature distributions and evaluated how varying tubular patterns affect ECC during pause-induced calcium release and rapid pacing. In our simulations, atrial myocytes with sparse and irregular tubules exhibit greater vulnerability to arrhythmia (e.g., shorter latency and higher amplitude for delayed afterdepolarizations, lower pacing threshold for alternans). Tubular remodeling alters the distribution and spatial/functional association of calcium-related transporters and increases whole-cell calcium load by reducing both ICaL inactivation in systole and Na/Ca exchange-mediated calcium extrusion in diastole. We have established a novel framework to mechanistically link ultrastructural remodeling to cellular instabilities. Novel insight from our model-based analysis could inform future therapeutic anti-AF strategies targeting structural remodeling.

Molecular simulations and enhanced sampling for the permeation of short peptides across a model of the skin barrier

Lanie Ruiz-Perez[a], Carlo Martinotti[a], Eveline Deplazes[b], and Ricardo Mancera[a]
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[b] *School of Life Sciences, University of Technology Sydney, Ultimo, NSW 2007 Australia.*

In the context of dermal and transdermal drug delivery there is great interest in predicting drug candidates with faster permeation rates across the stratum corneum (SC), the outer layer of the skin. The intercellular environment of the SC features a series of stacked lipid bilayers (LBs) in the gel phase. The ceramide-rich composition and high dehydration state of these LBs give rise to the barrier function of the skin due to their remarkable resistance to permeation of exogenous compounds. One experimental approach to enhance transdermal delivery is the chemical modification of the drug candidates. In particular, the lipid conjugation of short peptides shows promising results.
The slow dynamics of LBs in the gel phase hampers the accuracy of permeability estimates from molecular dynamics simulations, requiring the application of enhanced sampling methods. In this context, conventional umbrella sampling (US) yields insufficient sampling because as the drug candidate interacts with the LB, it visits only a reduced space of configurations. Insufficient sampling translates into inaccurate free energies of permeation and permeability coefficients for the drug candidates.
We employed the selective replica exchange with solute tempering (REST3) in combination with US. The novel REST3 enables the selective scaling of solute-solvent, membrane-solvent and membrane-membrane non-bonded interactions. We have applied the REST3+US combination to characterise the permeation of a set of short peptides and their lipid conjugates across a model LB representing the SC.
In comparison to conventional US, REST3+US converges faster and yields broader sampling of configuration of the Ala-Trp dipeptide. For the lipid conjugated short peptides, the predicted free energy profiles are in agreement with the experimental observations regarding their transdermal permeability.

Comprehensive preclinical cardiac safety evaluation of potential COVID-19 drugs

Clifford TeBay BSc[a*], Jeffrey R. McArthur PhD[a,b*], Melissa Mangala PhD[a,c], Nicholas Kerr MBBS[a,c], Stewart Heitmann PhD[1], Matthew D. Perry PhD[a,d], Monique J. Windley PhD[a,c], Jason C Kovacic MBBS PhD[a,c,e], Jamie I Vandenberg MBBS PhD[a,c], Adam P. Hill PhD[a,c]

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d. School of Medical Sciences, UNSW Sydney, Sydney, Australia.

e. Icahn School of Medicine at Mount Sinai, NY, USA.

Hydroxychloroquine and chloroquine, alone or in combination with azithromycin, are proposed as therapies for COVID-19. However, there is currently scant and inconsistent data regarding their proarrhythmic potential specifically in the context of these patients to guide clinical decision making. Moreover, their risk profile in the setting of altered physiological states encountered in patients with COVID-19 (i.e. febrile state and/or electrolyte imbalances) are unknown. As such, the aim was to characterise the cardiac safety profiles of hydroxychloroquine, chloroquine and azithromycin and their modification by COVID-19-associated metabolic changes including febrile temperature, acidosis and electrolyte imbalances.

Potency of hERG block, the molecular target for drug-induced arrhythmia, was measured using high-throughput electrophysiology. These potencies informed simulations to predict population risk profiles. Chloroquine and hydroxychloroquine blocked hERG with an IC_{50} of 1.47±0.07 μM and 3.78±0.17 μM respectively, indicating proarrhythmic risk at concentrations effective against SARS-CoV-2 *in vitro* and proposed in COVID-19 clinical trials. Hypokalaemia and hypermagnesemia increased potency of chloroquine and hydroxychloroquine, indicating increased proarrhythmic risk. Acidosis significantly reduced potency of all drugs (i.e. reduced proarrhythmic risk), whereas increased temperature decreased potency of chloroquine and hydroxychloroquine but increased potency for azithromycin. *In silico* simulations across genetically diverse populations predicted that 17% of individuals exhibit action potential durations >500 ms at the highest proposed therapeutic levels, equating to significant QT prolongation.

Significant proarrhythmic risk is predicted for hydroxychloroquine and chloroquine at doses proposed to treat COVID-19. Clinicians should carefully consider the risk of such treatments, and implement long term QT interval monitoring in trials, particularly in patients with electrolyte imbalances.

Models of muscle contraction must include force-dependent weakly-bound cross-bridges and series elasticity to describe stretch-activation in *Dropsophila* jump muscle fibers

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[d] *Department of Mathematical Sciences, Worcester Polytechnic Institute, MA, USA.*

Stretch-activation (SA) is a cellular phenomenon in muscle that is inherent in most muscle types. While it has been shown experimentally that SA plays a significant role in processes like insect flight and heart contraction, the molecular mechanisms responsible for this phenomenon are unknown [1]. SA is observed in the tension transient response of a muscle fiber after a small, rapid stretch. Experimentally, a muscle fiber is calcium-activated and held isometrically, and then a small, rapid stretch is applied. The typical tension transient response is a rapid increase in tension at the time of stretch, a subsequent rapid decay, followed by a delayed increase in tension, known as stretch-activation. To understand the mechanism responsible for SA, we developed a minimal cross-bridge model and quantitatively compared it to two complementary measurements from *Drosophila* jump muscle fibers: 1) tension transients after a rapid stretch, which evoke a stretch-activation response, and 2) steady-state force-velocity measurements. We find that a minimal cross-bridge model including two unbound states, two strongly-bound (and thus force-producing) states, and one weakly-bound (and thus non-force-producing) state, is unable to simultaneously describe our measurements performed on the same muscle type and under the same conditions. However, if we additionally include force-dependent detachment from the weakly-bound state and a linearly elastic element in series with the cross-bridges, the model is able to simultaneously describe both tension transient and force-velocity data.

The ability of this cross-bridge model to capture fiber level behavior provides insight into the molecular mechanisms of these larger-scale phenomena. Notably, our model successfully replicates stretch-activation, suggesting that the molecular mechanism for SA is a result of the interaction between the cross-bridges and an elastic element in series. We find that this interaction is necessary for successful model fits, highlighting the complex connection between fiber-level tension transients and molecular-level dynamics. Finally, our model results suggest that the curvature of the force-velocity relationship is determined by the force-dependence of both strongly- and weakly-bound cross-bridges, providing new perspective to a well-studied characteristic of muscle.

[1] R.K. Josephson, J.G. Malamud, D.R. Stokes, Asynchronous muscle: a primer, Journal of Experimental Biology 203 (18) (2000) 2713 – 2722.

In-cell Structure Determination of an Antimicrobial Peptide by DNP solid-state NMR

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As bacteria develop increasing resistance to antibiotics, antimicrobial peptides (AMPs) are possible alternatives to conventional treatments. Maculatin 1.1 (Mac1) is a cationic AMP isolated from the skin glands of the Australian tree frog *Litoria genimaculata* with low micromolar activity against Gram-positive bacteria.[1] The molecular mechanism of Mac1 has been well studied *in vitro* but there is a scarcity of info *in vivo* studies. [2-3] Solid-state nuclear magnetic resonance (ss-NMR) is a valuable tool to investigate peptide-membrane interactions but its application *in vivo* is restricted due to its inherent insensitivity and short lifespan of bacteria. However, Dynamic Nuclear Polarization (DNP) NMR enables in-cell experiments due to enhanced sensitivity and longer cell survival at the low temperatures used. Free electrons or spin labels together with isotope enriched peptides are needed for DNP-NMR structural studies. Hence, we designed mono- and bi-radical nitroxide (TOAC) spin-labelled peptides, TOAC-MacW and TOAC-TOAC-MacW, as a vector to locate radical sources within cell membranes. In-cell NMR results showed that mono- and bi-TOAC radicals induced more localized ^{13}C signal enhancement compared to the more hydrophilic radical AMUPol.[4] Circular dichroism and solution NMR spectroscopy revealed that the spin-labelled peptides adopt a helical conformation in lipid environments. We then used an *E. coli*-based expression method to achieve isotope labelling of Mac1 with C-amidation to facilitate site-specific interrogation of structure and intermolecular contacts. A protocol for expression and purification of milligram quantities of amidated and uniformly ^{15}N labelled Mac1 will be presented.

[1] B.C.S. Chia, J.A. Carver, T.D. Mulhern, J.H. Bowie, *Eur*. *J*. *Biochem.* **267**, 1894-1908 (2000).
[2] M.-A. Sani, F. Separovic, *Acc. Chem. Res.* **49**, 1130-1138 (2016).
[3] M.-A. Sani, T. C. Whitwell, F. Separovic, *Biochim*. *Biophys*. *Acta* **1818**, 205-211 (2012).
[4] M.-A. Sani, S. Zhu, V. Hofferek, F. Separovic. Nitroxide spin labelled peptides for DNP-NMR in-cell studies. *FASEB J*. **33**, 000-000. (2019)

Structural basis for the human/mouse species selectivity of 6-substituted 5-(hexamethylene)amiloride (HMA) analogs as inhibitors of the urokinase plasminogen activator

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Urokinase plasminogen activator (uPA) plays a critical role in tumour cell invasion, migration and metastatic dissemination and is a promising target for the development of anti-metastasis drugs. 6-Substituted analogs of 5-(hexamethylene)amiloride (HMA) are potent uPA inhibitors that show anti-metastatic effects *in vivo*, high selectivity over closely related trypsin-like serine proteases without the diuretic and anti-kaliuretic properties of amiloride [1,2]. However, the compounds display a distinct selectivity for human over mouse uPA, thus confounding interpretation of data from human xenograft mouse cancer models. To understand the reasons for the species differences and support the design of dual-potent inhibitors for *in vivo* target-validation studies, we performed molecular dynamics simulations (MD) and alchemical free energy perturbation (FEP) calculations on human, partially murinised human (H99Y) and mouse uPA and their complexes with amiloride, HMA and four 6-substituted HMA analogs.

The simulations were able to recapitulate the experimental uPA inhibitory potencies for amiloride, HMA and the 6-substituted HMA analogs. Residue 99, which is different in human (His) and mouse (Tyr) uPA, appears to be a key residue that contributes to loss of affinity for mouse uPA. The structural insights gained from the study guided us towards a particular HMA analog in our library, which was shown to have increased potency for mouse uPA due to improved interactions with Glu146 in the S1β pocket.

[1] B. J. Buckley, A. Aboelela, E. Minaei, L. X. Jiang, Z. Xu,; U. Ali, K. Fildes, C.-Y. Cheung, S. M. Cook, D. C. Johnson, D. A. Bachovchin, G. M. Cook, M. Apte, M. Huang, M. Ranson, M. J. Kelso, 6-Substituted Hexamethylene Amiloride (HMA) Derivatives as Potent and Selective Inhibitors of the Human Urokinase Plasminogen Activator for Use in Cancer. *J. Med. Chem.*, **61(18)**, 8299 (2018).
[2] B. J. Buckley, H. Majed, A. Aboelela, E. Minaei, L. Jiang, K. Fildes, C.-Y. Cheung, D. Johnson, D. Bachovchin, G. M. Cook, 6-Substituted amiloride derivatives as inhibitors of the urokinase-type plasminogen activator for use in metastatic disease. *Bioorg. Med. Chem. Lett.*, **29(24)**,126753 (2019).

Understanding the concentration-dependent Interaction of Sterol Drugs with Model Lung Surfactant Monolayers Using Molecular Dynamics Simulations

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The lung surfactant monolayer (LSM) is the main barrier for particles entering the lung, including steroid drugs used to treat lung diseases. Understanding the molecular interaction of steroid drugs with the LSM is critical for effective dosing and delivery of existing drugs, as well as the rational design of new sterol drugs. However, the molecular-level mechanism of how drugs diffuse into the LSM is poorly understood. Part of that is a lack of molecular models that mimic the physicochemical properties of the LSM. In this study, biomolecular simulations are carried out to investigate the molecular interactions of sterol drug molecules with the LSM. Specifically, coarse-grained (CG) molecular dynamics (MD) simulations are used to understand the concentration-dependent interaction of the clinically relevant sterol drugs prednisolone under different breathing conditions (inhalation and exhalation). The LSM is composed of the zwitterionic lipids DPPC and POPC as well as cholesterol. Our results show that at drug concentrations of <3.0% w/w, the drug has only a slight effect on the structure of the LSM during both breathing conditions, while at concentrations of 3.0% w/w, the presence of the drug reduces the are aper lipid and alters lipid order parameters. At high concentration (≥5.9% w/w), the drug causes a collapse of the monolayer. Analysis has showed that the collapse is likely caused by the formation of clusters and an inability of the drug to diffuse in the LSM. Overall, the monolayer is most susceptible to a drug-induced collapse during exhalation in the presence of cholesterol, while the presence of the negatively charged lipid POPG appears to have a protective effect. The findings of this investigation will be helpful to understand the interaction between sterol drugs and lung surfactants better, in particular how altering the spreading mechanism might be used to prevent monolayer collapse.

Separation and enrichment of sodium-motile bacteria using cost-effective microfluidics

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4. *CSIRO Future Science Platform for Synthetic Biology*

Sodium-motility has significant medical relevance since sodium-powered pathogens such as *Vibrio cholerae*, which has up to 4M cases per year, strictly require sodium ions to drive motility [1]. For the most part, bacterial motility is driven by a molecular motor called the bacterial flagellar motor (BFM). The development of cost-effective techniques to separate bacteria more efficiently will enable us to examine subtle differences in bacterial phenotype. Microfluidics platforms are emerging as a technology with wide promise for microbiological research [2], since they offer precise control of flow and buffer composition at the micron scales that bacteria inhabit.

Here, we fabricated a simple microfluidic device with a Y-shaped millimetre-sized channel. We demonstrated efficient separation and enrichment of sodium-motile bacteria (*Escherichia coli*) by generating stable laminar-flow of two streams using Arduino-controlled 3D-printed syringe pumps. Using this device, we were able to distinguish and separate sodium-motile bacteria in solution. We were able to tune the separation of motile bacteria from a sodium-free solution due to the controlled diffusion of sodium ions across the laminar flow interface. Lastly, we showed enrichment of sodium-motile bacteria from 10% to ~ 75% when running a mixed population of motile and non-motile bacteria through our microfluidic device.

Overall, microfluidics allows fine separation of cells based on subtle differences in motility traits with applications in synthetic biology, directed evolution, and applied medical microbiology.

[1] C. C. Häse and B. Barquera, “Role of sodium bioenergetics in Vibrio cholerae,” *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, vol. 1505, no. 1, pp. 169–178, May 2001, doi: 10.1016/S0005-2728(00)00286-3.

[2] O. Scheler, W. Postek, and P. Garstecki, “Recent developments of microfluidics as a tool for biotechnology and microbiology,” *Current Opinion in Biotechnology*, vol. 55, pp. 60–67, Feb. 2019, doi: 10.1016/j.copbio.2018.08.004.

Session 1a: Ion channels and pumps

13:00-14:45

Saturation mutagenesis study of heterozygous hERG channel variants using SyncroPatch 384

Chai-Ann Ng[a,b], Jess Farr[a], Adam P. Hill[a,b] and Jamie I. Vandenberg[a,b]
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The expression of hERG potassium channels at the plasma membrane of cardiac myocytes is critical for the coordinated propagation of the electrical signals that regulate the rhythm of the heartbeat. Reduced hERG function due to mutations in *KCNH2*, that cause long QT syndrome type 2 (LQTS2), increases the risk of sudden cardiac arrest. Mutations may affect mRNA or protein subunit synthesis, assembly of subunits into the functional tetrameric channels, trafficking and/or gating and ion permeation of the channels once they have reached the plasma membrane. To assess the impact of any variant in *KCNH2*, the ideal method is patch-clamp electrophysiology as it can simultaneously assess levels of expression as well as gating and ion permeation.

We have developed a high throughput functional phenotyping assay that could quantify the extent of loss-of-function of *KCNH2* variants using automated patch-clamp electrophysiology platform (SyncroPatch 384PE) in conjunction with heterozygous stable Flp-In HEK293 cell lines that co-express mutant with WT *KCNH2* alleles. A proof of concept study for 30 variants has been published [1].

Here, we have combined large scale functional phenotyping with saturation mutagenesis to characterise all possible 458 missense single nucleotide variants in exon 2 of *KCNH2*, to determine which residues are intolerant to mutations. This data can be used to assess the accuracy of bioinformatic predictions and will form the basis of a database for functional phenotyping of all *KCNH2* variants.

The current density measurement for these 458 *KCNH2* variants largely correlates to the trafficking efficiency of the hERG channel to the plasma membrane. The amount of available current density correlated well with the secondary structure of the hERG protein encoded by exon 2. An analysis of sequence conservation among *KCNH* family of ion channels reveals certain residues are 100% conserved and intolerant to mutations. On the other hand, 71 out of 76 *KCNH2* exon 2 variants (~15%) that occurred in residues that are not conserved across the *KCNHx* ion channel family displayed normal current densities. Our functional phenotyping result can also help the classification of variant pathogenicity.

In the era of precision medicine, large numbers of *KCNH2* variants with uncertain significance are going to be identified. Our high-throughput functional phenotyping assay will not only allow identification of high impact variants, it can also be used to filter out non-pathogenic variants.

[1] Ng et. al. High-throughput phenotyping of heteromeric human ether-à-go-go-related gene potassium channel variants can discriminate pathogenic from rare benign variants. Heart rhythm. **17** (3), 492-500 (2020).

What is the origin of the upside down kinetics of HERG K^+ channels

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Human ether-a-go-go-related gene (hERG) K^+ channels play a critical role in regulating cardiac electrical excitability. Like other voltage-gated potassium (VGK) channels, hERG channels open and close in response to changes in membrane voltage and undergo inactivation in the presence of prolonged membrane depolarizations. Inactivation in hERG channels occurs via a process known as "collapse" of the selectivity filter, which has been well described in a range of different potassium channels [1]. Unlike in other channels however, inactivation in hERG channels is much more rapid than the activation/deactivation process. Furthermore, inactivation in hERG is intrinsically voltage dependent, which is critical to the role it plays in shaping the cardiac action potential, and mutations that either reduce or enhance the voltage sensitivity of hERG inactivation greatly increase the risk of cardiac arrhythmias [2].

The aim of this study was to investigate the origin of the voltage dependence of inactivation in hERG channels. HERG channels were expressed in xenopus oocytes and rates of inactivation / recovery from inactivation were measured at voltages in the range +80 to +160 mV using two-electrode voltage clamp technique.

In WT hERG channels, perfused with extracellular solution containing 1 mM K^+, the observed rates of inactivation decreased from 982 ± 133 s-1 (mean ± SD, n=11) at +80 mV to 152 ± 23 s^{-1} at 0 mV. Moderate increases in extracellular K^+ caused more significant slowing of inactivation at less depolarized voltages compared to more depolarized voltages. This suggests that at more positive voltages it is harder for K^+ to bind to the site that slows inactivation. To test the hypothesis that K^+ modulation of the voltage dependence of inactivation was caused by K^+ binding to a site that sensed the membrane electric field across the channel we measured the apparent affinity for K^+ slowing of inactivation at voltages in the range 0 to +60 mV and fitted the data with the Woodhull equation:

$$IC_{50} = IC_{50}(0mV) \cdot \exp(z\delta \cdot V_m \cdot e/kT) \qquad \text{(Eqn. 1)}$$

where z, is the charge on the particle, δ is the fraction of the voltage field sensed by the blocker from the outside of the membrane and e, k, T have their usual thermodyanimc meanings.

If we assume that a single K^+ site is being titrated (i.e., z=+1 in Eqn 1), then the value for δ is 0.46. How this could be interpreted in light of collapse of the selectivity filter in hERG channels will be discussed.

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[2] Vandenberg JI et al. (2012) HERG K+ channels: Structure, Function and Clinical Significance. Physiol Rev 92: 1393-1478

Piezo1 channels as regulators of integrin-mediated focal adhesions in cardiac fibroblasts

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Integrin-mediated focal adhesions form the primary link between the extracellular matrix (ECM) and the cytoskeleton and are critical subcellular sites for sensing substrate stiffness and ECM composition [1]. These properties of the cellular micro-environment, in addition to exogenous forces (i.e. stretch), are key modulators of cardiac fibroblast phenotype and function. Piezo1 is a calcium permeable ion channel and well-established key sensor of mechanical forces in the cardiovascular system [2]. In this study, we aimed to understand whether crosstalk exists between Piezo1 and integrin-mediated focal adhesions.

Using Total Internal Reflection Fluorescence (TIRF) Microscopy we observed a high level of colocalization of Piezo1 with integrin-mediated focal adhesions on different ECM matrices; an observation that was corroborated in different cell types, across different lineages. Consistent with this localization we observed loss of Piezo1 from focal adhesions as they were broken down in response to Myosin II inhibition or Rho-kinase blockade. Knock down of Piezo1 with siRNA led to a reduction in focal adhesion size consistent with a role in adhesion maturation. Chemical activation of Piezo1 using the agonist Yoda-1 caused β-integrin sub-type dependent changes in focal adhesions and produced effects indicative of fibroblast activation/differentiation. Furthermore, Piezo1 levels were responsive to substrate stiffness and its sub-cellular localization was drastically altered by TGF-beta1; the master regulator of fibrotic remodelling.

These results highlight the possibility that Piezo1 may play a role in cardiac fibrotic remodelling via focal adhesion signalling (turnover and maturation) in cardiac fibroblasts.

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Screening for novel sodium motility inhibitor

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The bacterial flagellar motor (BFM) is a protein complex driving motility of cells. The BFM consists of a rotor, a filament and the stators. The stators are ion-selective transmembrane channel protein, converting electrical energy into torque to drive the rotation of the motor. The BFM imparts motility and in turn, contributes to the survival of the cell and virulence of many pathogens. The most common ionic power sources of BFM are protons (H^+) and sodium ions (Na^+). The sodium powered stators, such as those in the PomAB stator complex of *Vibrio* spp, are essentially sodium ion channels and thus can be inhibited by channel inhibitors. Such inhibition of motility has assisted in the discovery of many of the mechanisms underlying flagellar driven motility. In particular, phenamil is a potent and widely used inhibitor for sodium-coupled stators but, overall, there have not been many new motility inhibitors since its discovery in 1988. In this study, we discovered a novel motility inhibitor HM2-16F from a small library of amiloride derivatives. Using our single-cell tracked tethered cell assay, we showed that HM2-16F had comparable inhibition to phenamil. We demonstrated that HM2-16F was a reversible non-competitive inhibitor of the Na^+ coupled PomA PotB stator complex. We further showed that HM2-16F could inhibit H^+ driven MotAB stator complex via an unspecific interaction. While the mechanism of this MotAB inhibition is unknown, HM2-16F was shown to inhibit motility in multiple different assays and on multiple stator types. With the further characterisation of HM2-16F to determine the mechanism of inhibition, we may be able to deliver a novel compound for studying the flagellar motor and for providing anti-virulence effect to combat rising antimicrobial resistance.

The Role of Epithelial Sodium Channel (ENaC) and Endothelial Glycocalyx in Influencing Mechanical Properties of Endothelial Cells.

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Background: Increased endothelial cell stiffness (ECS) is an important determinant of hypertension that coincides with altered endothelial nitric oxide (NO) production and endothelial dysfunction. Endothelial dysfunction is associated with degraded endothelial glycocalyx and elevated ENaC. However, the mechanism behind stiffness in not well studied. I hypothesize F-actin, a major cell cytoskeleton element as a key determinant to influence mechanical properties of endothelial cells.

Approach: ENaC expression was changed by aldosterone treatment in Human Umbilical Vein Endothelial Cells (HUVECs) and was detected by immunoblotting. ECS was assessed by Atomic Force Microscopy (AFM) in fixed cells. F-actin changes were assessed through immunofluorescence microscopy. Wheat germ agglutinin (WGA) was used to detect glycocalyx in untreated and heparinise III treated cells. ECS in these cells was also measured by AFM.

Results: Aldosterone significantly increased α- ($p<0.05$), and δ-ENaC ($p<0.05$) protein levels. The elevated ENaC expression was associated in increased Young's modulus ($p<0.0001$), indicating increased ECS. Interestingly, these changes were associated with increased F-actin ($p<0.01$). The use of Texas Red–conjugated WGA showed detectable fluorescence for the endothelial glycocalyx. Treatment with heparinase III resulted in decreased WGA staining indicating successful degradation of the glycocalyx. Moreover, the treatment with heparinise III resulted in increased ECS ($p<0.05$).

Conclusions: Overall, our study provides experimental evidence of increased F-actin as a key factor to influence mechanical properties of endothelial cells. This is a new mechanism through which ENaC in endothelial cells regulates ECS to contribute to endothelial dysfunction and hypertension.

Purification and structural characterization of TACAN, a novel ion channel implicated in pain sensing

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TACAN (Tension-Activated-ChANnel) has recently been identified as one of the principle pain-sensing channels via mechanotransduction which is referred to the conversion of physical forces into electrical and biochemical signals [1]. TACAN does not share significant sequence similarity to any known class of ion channel and thus represents a novel class of mechanosensitive channels. Presently, the three-dimensional structure of TACAN and its gating mechanism are unknown. Based on bioinformatics sequence analysis, TACAN is expected to comprise 5 or 6 transmembrane helices, a 138 residue N-terminal domain and a 14 residue C-terminal domain that includes a cluster of 9 basic residues. We have been successful in purifying TACAN using three different phospholipid membrane mimetic system, such as detergent (DDM) and peptidisc and Saposin lipid nanoparticles (Salipro). TACAN purified in all three systems was used for cryo-EM. TACAN-Salipro has been the most promising for high resolution data collection. TACAN-Salipro incorporation was optimised and high resolution cryo-EM data was collected on purified fractions. Image processing and 3D reconstruction are in progress. This study will help obtain knowledge about the structure and architecture of the transmembrane domain, the importance of the N-terminal and C-terminal domains in channel function and the essential residues lining the pore-forming helices. The structure of TACAN is essential to understanding the underlying mechanisms of function of this channel which will increase our knowledge of channel structure and open the path to developing potential inhibitors and drugs for chronic pain.

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Understanding the structural basis of inactivation mechanism of the hERG potassium channel

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The human ether-a-go-go related gene (hERG) potassium ion channel carries the major repolarising current in the cardiac action potential. Loss-of-function mutations of hERG result in prolongation of the cardiac QT interval and increase the risk of cardiac arrhythmias and sudden cardiac death. Like other voltage-gated potassium (VGK) channels, hERG channels can exist in closed, open, and inactivated states. However, the gating kinetics of hERG channels are different from other VGK channels. For instance, transitions between open and closed states are unusually slow, whereas transitions between the open and inactivated states are both very fast and voltage dependent. The first structure of the hERG channel was determined in the open conformation and much of the molecular details on gating remains unknown. We have used cryo-electron microscopy (cryo-EM) to further elucidate the mechanism of channel inactivation of the hERG potassium ion channel. In previous functional studies we showed that loss of K^+ from the selectivity filter was important for the transition from the open to inactivated state [1]. Here, we have determined structures of the hERG channel in the presence of 3 mM and 300 mM K^+. We have observed a collapsed selectivity filter in the low K^+ structure with a constriction at F627. The diameter of the pore at F627 was reduced from 2.4 Å to 1.2 Å. We will discuss and compare the open and inactivated structures in more detail.

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Session 1b: Computational Biophysics

Membrane Tension Can Enhance Adaptation to Maintain Polarity of Migrating Cells

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Migratory cells are known to adapt to environments that contain wide-ranging levels of chemoattractant. Although biochemical models of adaptation have been previously proposed, here, we discuss a different mechanism based on mechanosensing, in which the interaction between biochemical signaling and cell tension facilitates adaptation. We describe and analyze a model of mechanochemical-based adaptation coupling a mechanics-based physical model of cell tension coupled with the wave-pinning reaction-diffusion model for Rac GTPase activity. The mathematical analysis of this model, simulations of a simplified one-dimensional cell geometry, and two-dimensional finite element simulations of deforming cells reveal that as a cell protrudes under the influence of high stimulation levels, tension-mediated inhibition of Rac signaling causes the cell to polarize even when initially overstimulated. Specifically, tension-mediated inhibition of Rac activation, which has been experimentally observed in recent years, facilitates this adaptation by countering the high levels of environmental stimulation. These results demonstrate how tension-related mechanosensing may provide an alternative (and potentially complementary) mechanism for cell adaptation [1].

[1] C. Zmurchok, J. Collette, V. Rajagopal, and W. R. Holmes, “Membrane Tension Can Enhance Adaptation to Maintain Polarity of Migrating Cells,” *Biophysical Journal*, vol. 119, no. 8, pp. 1617–1629, Oct. 2020, doi: 10.1016/j.bpj.2020.08.035.

Conformational Determinants of Cyclic Peptide Permeability

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Peptide passive membrane permeability is important to oral delivery of peptide pharmaceuticals. The general mechanism of peptide passive permeability remains unclear. Here, we investigated the relationship between conformation and permeability for a series of cyclic hexapeptides that have a wide variety of PAMPA permeabilities, despite only differing in residue chirality. Using enhanced sampling molecular dynamics simulations, we studied the populations of peptide conformations common to both polar and nonpolar solvents. Utilizing a conformational selection-like model of peptide permeability, we were able to correlate the populations of conformations common to both polar and nonpolar solvents with PAMPA permeability. Moreover, we validated our enhanced sampling simulations against in-house nuclear magnetic resonance spectroscopy structures of the peptides. We anticipate that our enhanced sampling molecular dynamics simulation method of predicting peptide permeability may be applicable to other cyclic peptide scaffolds.

Host-derived lipids impact the membrane dynamics, antibiotic resistance evolution and efflux systems of *Acinetobacter baumannii*

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[b] *Molecular Sciences and Technology, Flinders University, South Australia, 5042, Australia*

Acinetobacter baumannii is one of the world's most problematic nosocomial pathogens and is rapidly acquiring resistance to a range of frontline antibiotics. Despite mediating antibiotic entry into the cell, our understanding of the *A. baumannii* membrane composition and its impact on antibiotic resistance is limited. This is of particular importance, as membrane composition can vary in response to growth environment and the acquisition of host-derived lipids, some of which function directly as antimicrobial agents. In this study, we use molecular dynamics simulations to determine the biophysical properties of the *A. baumannii* membrane following the incorporation of host-derived polyunsaturated fatty acids (PUFAs). Changes in membrane ordering and dynamics were observed and were associated with increased antibiotic susceptibility and a reduced ability to gain resistance. In *A. baumanii* resistance to many common antibiotics is mediated by bacterial resistance-nodulation-division (RND) transporter systems, such as AdeABC and AdeIJK, which efflux antimicrobials from the cell. Functional analyses of primary *A. baumannii* efflux systems indicated AdeB-mediated resistance was impacted by PUFAs. Molecular dynamics simulations of AdeB identified altered conformational cycling in a PUFA-enriched membrane, providing a structural basis for enhanced antimicrobial susceptibility following PUFA treatment. Collectively, we have shown that *A. baumanii* membrane dynamics are modulated by host-derived PUFAs and that this modulation can impact antibiotic efficacy and microbial resistance evolution through a relationship with RND efflux pumps.

Bak Core Domain Dimers Alter Membrane Dynamics; Assessed by Atomistic and Coarse-Grain Simulations

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The programmed cell death process apoptosis is mediated in part by members of the Bcl-2 family, Bak and Bax [1]. Activation of these pro-apoptotic members leads to mitochondrial outer membrane permeabilization (MOMP) and subsequently is seen as the 'point of no return' for the cell lifecycle [2]. Although well understood that both Bak and Bax form symmetric dimers and further oligomeric structures on the MOM, high resolution structural elucidation of these oligomeric structures and the nature of the resultant proteolipidic pore structure is still unknown [3].

Using a recently described crystal structure of a Bak core domain dimer, molecular dynamics simulation of the dimer embedded in an all-atom membrane identified that helices within the core interact directly with lipid acyl chains, producing significant curvature in, and thinning of the membrane. Such curvature and stressing of membrane, altering membrane tension is thought to be a key driver in MOMP [4,5]. Further, where targeted mutagenesis of key residues has been found to impair membrane permeabilization, we demonstrate computationally the mechanism by which this occurs, decreasing this aforementioned membrane thinning. We further identify using a coarse-grain representation of the MOM and Bak dimer, the bilayer self-assembly process and subsequent interaction of the dimer over the microsecond time scale.

Collectively, this work provides the first molecular details of activated Bak dimers interacting with lipid molecules at the bilayer surface, and provides evidence to suggest that indeed the membrane itself plays a role in stabilisation of the oligomer.

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Shape Effects of Gold Nanoparticles on Model Lung Surfactant Monolayer

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The rapid development of nanotechnology increases the huge aspects of Nanoparticles (NPs) in biomedical applications particularly gold nanoparticles (AuNPs) with the potential health risk associated in instigation of lungs diseases. Being nanoscale size, the inhaled NPs varies of different sizes and shapes and primarily interact with lung surfactant (LS) monolayer in the lungs alveoli. The LS monolayer is a thin lipoprotein layer at the gas-exchange interface inside alveoli which bolsters easy gas-exchange process during breathing. Gold NPs (AuNPs) are proved as harmful pollutants in the occupational circumstances such as mining and processing of gold/gold made products. Coarse-grained molecular dynamics simulation has been carried out to investigate the shape effects of AuNPs on LS monolayer during breathing. Two extreme breathing conditions (inhalation and exhalation) have been considered to mimic the physiological breathing environment. Four different shapes (spherical, icosahedron, rod, and box) of AuNPs are considered to detect the individual shape effect on LS monolayer's structural and dynamical properties. Upon deposition of AuNPs (all shapes), the NPs perturb the LS monolayer typical structural and obstruct the physical movement of surfactant components (lipids and peptides). These structural and dynamical changes in LS monolayer's components induce by AuNPs are more observable in the compressed monolayer than an expanded monolayer. We have observed that surfactant peptide are less diffuse in the monolayer exposed to box AuNPs compared to other shapes. Our simulations also show that the presence of different shapes AuNPs significantly affects the clustering of surfactant cholesterol/peptides and phospholipid arrangement in the monolayer. Compared to other surfactant molecules, cholesterol adsorption to AuNPs surface is higher particularly for spherical AuNP at monolayer breathing conditions. Overall, our results will assist in accessing the shape-based NPs on the biophysical properties of the lungs during breathing which could be used in evaluating the shaped-based NPs roles of lung diseases.

An integrative approach to understand the transport of ions across phospholipid bilayers.

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In this talk we will present the results from two recent studies that demonstrate how molecular dynamics (MD) simulations combined with tethered bilayer lipid membrane (tBLM) experiments can be used to understand, in molecular detail, how ions are transported across membranes.

The first study is focused on understanding the permeation of alkali ions across phospholipid bilayer. Specifically, we investigate ion-induced pore formation by Na^+ and K^+ ions and how this is related to differences in their membrane permeability and changes in the structure of the phospholipid bilayer [1]. Data from tBLM/EIS experiments using K^+ and Na^+ with phospholipid bilayers composed of different lipids as well as MD simulations reveal that differences in membrane permeability originate from distinct ion coordination by the carbonyl oxygen in the lipid headgroup, altering the propensity for bilayer formation. We propose a new model of ion permeation based on ion-induced defects that are not solely based on the affinity the cation for the membrane. This model suggests that the differences between the permeation of cations across phospholipid bilayers is driven by the ability of the cations to condense lipids that then resist pore formation. More recent experiments show that this model also explains the low membrane permeability of Ca^{2+} (unpublished).

In the second study, we use tBLM/EIS and MD simulations to characterise the structure and cation-selectivity of ion channels created by pore-forming peptides [2]. While the ability of viral fusion or antimicrobial peptides to form ion-conducting pores is well documented, the structures of these pores are poorly defined. We characterize the size and multimeric structure of pores formed by GALA; a 30-reisdue peptide, pH-sensitive peptide. Specifically, we used a number of alkaline earth cations and the large organic cations, choline and triethanolamine, to estimate the pore size by measuring the concentration-dependent changes in membrane conduction. This data was combined with MD simulations to estimate the multimeric state of GALA pores. We report that in planar phospholipid bilayers, GALA pores likely consist of six peptide monomers rather than eight to twelve monomers, as previously reported. We further demonstrate, for the first time, that GALA exhibits cation selectivity, which is based on $\Delta G_{hydration}$ of the ions. This integrative approach provides a powerful approach to characterise the structure and ion-selectivity of a wide range of pore-forming peptides.

[1] Deplazes, E., et al., *Role of Ion–Phospholipid Interactions in Zwitterionic Phospholipid Bilayer Ion Permeation.* The Journal of Physical Chemistry Letters, 2020: p. 6353-6358.

[2] Deplazes, E., et al., *Structural Characterization of a Cation-Selective, Self-Assembled Peptide Pore in Planar Phospholipid Bilayers.* The Journal of Physical Chemistry Letters, 2020. **11**(19): p. 8152-8156.

Evaluation of the Intermolecular Interactions between the Peptide-based Inhibitors and Amyloid-β Monomer/Fibril Structures

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Alzheimer's disease (AD) is a neuro-irreversible disorder that progresses gradually and impairs the life quality of patients through progressive memory loss, behavioral changes [1]. It is reported that conformation conversion of α-helix to β-sheet in amyloid-beta (Aβ) peptide monomers and ultimately the formation of the Aβ aggregations have the main role in AD appearing and progression [2]. Recent studies demonstrated that the strategy of peptide-based inhibitors could be considered as a novel approach for AD therapy [3]. Therefore, in recent years, the rational design of effective inhibitory compounds provides the employing necessity of computational approaches for a better understanding of intermolecular interactions between the ligand-target complex [4, 5].

With respect to these findings, we evaluated intermolecular interactions between a series of derived peptides of $A\beta_{31-36}$ sequence, based on previous reported peptide LIAIMA [6], and Aβ monomer and fibril structures using molecular docking. Molecular docking studies of the designed peptides were successfully carried out onto the $Aß_{42}$ monomer and fibril structures using AutoDock Vina software. The $Aß_{42}$ monomer and fibril structures are prepared from the Protein Data Bank with code 1IYT and 2BEG respectively. The designed peptides also were drawn and minimized by MarvinSketch and HyperChem respectively

The obtained findings demonstrated that designed peptides have a good affinity binding energy to Aβ monomer and fibril structures (a range from -3.8 to -4.3 kcal/mol for monomers and -4.3 to 5.8 kcal/mol for fibrils) in comparison with the peptide KVLFF as a reference which showed a binding energy of -4.7 and -6.3 kcal/mol for monomer and fibril respectively. They also could inhibit the conformation process of α-helix to β-sheet structure and the increasing growth of the Aβ aggregations.

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Session 2: Careers for Science Graduates
15:15- 17:15

Day 2: 3rd December 2020

Session 3: Australian Society for Biophysics/Biophysical Society of Japan joint session

10:00-12:20

Detect with PKAchu:Light-off-induced PKA activation in rod photoreceptor cells

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About: PKAchu: PKAchu is a fluorescent mouse designed to visualize the activity of cAMP dependent kinase (Protein kinase A, PKA; 'chu' being Japanese for 'squeak') [1]. PKAchu mice ubiquitously express AKAR3EV sensor protein, which changes fluorescence from cyan to yellow in response to the PKA activation [2]. PKAchu had been used for brain researches [3-4] and a neutrophil study [5], but not for the retina before I joined my current lab in 2017.

Background: The cAMP-PKA system in vertebrate visual photoreceptor cells, rods and cones, is light sensitive. Previous studies have shown that light stimulates extrasynaptic dopamine release in the retina, which is received by the G_i-coupled D4R dopamine receptor on photoreceptor cells to reduce cAMP and thus PKA activity. This PKA inhibition is thought to desensitize the photoreceptor cells, to form a negative feedback loop of the photosensitivity regulation (i.e. light sensitivity reduction by light). However, the spatio-temporal regulation of the light-induced PKA inhibition has not been well characterized due to technical limitations. Here, we tried two-photon PKA activity imaging of the photoreceptor cells to observe the PKA inhibition at single-cell resolution, in the order of seconds.

Methods: Ex vivo live imaging of the PKAchu retinal explant under a two-photon microscope.

Results: Unexpectedly, in addition to the aforementioned light-on-induced PKA inhibition, we detected a prominent light-off-induced PKA activation in photoreceptor cells [6]. The activation was confined to the illuminated rod photoreceptor cells among >140 subtypes of mouse retinal neurons. The spectral sensitivity of the PKA activation was very well fitted with an absorption spectrum of rhodopsin. Consistently, rhodopsin-deficient PKAchu and rhodopsin pathway-deficient PKAchu *Gnat1*$^{-/-}$ did not show the light-off-induced PKA activation.

Conclusion: Rod PKA is activated by light-off through an unknown rhodopsin-related pathway. This PKA activation is supposed to boost the dark-adaptation: the visual sensitivity enhancement in darkness.

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Modified N-linked glycosylation status predicts trafficking defective human Piezo1 channel mutations

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Mechanosensitive channels are integral membrane proteins that sense mechanical stimuli. Like all membrane proteins, they pass through biosynthetic quality control in the endoplasmic reticulum and Golgi that results in them reaching their destination at the plasma membrane. Here we show that N-linked glycosylation of two highly conserved asparagine residues in the 'cap' region of mechanosensitive Piezo1 channels are necessary for the mature protein to reach the plasma membrane. Both mutation of these asparagines and treatment with an enzyme that hydrolyses N-linked oligosaccharides (PNGaseF) eliminates the fully glycosylated mature Piezo1 protein. The N-glycans in the cap are a pre-requisite for higher-order glycosylation in the 'propeller' regions, which are present in loops that are essential for mechanotransduction. Importantly, trafficking-defective Piezo1 variants linked to generalized lymphatic dysplasia and bicuspid aortic valve display reduced fully N-glycosylated protein. The higher order glycosylation status *in vitro* correlates with efficient membrane trafficking and will aid in determining the functional impact of Piezo1 variants of unknown significance.

Improving two-photon excitation microscopy for deeper, sharper and/or faster intravital imaging

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Two-photon excitation laser scanning fluorescence microscopy (TPLSM) is a powerful tool to visualize intravital microstructures. This is because of its superior penetration depth and less-invasiveness in specimens owing to its near-infrared excitation laser wavelength compared with the single-photon excitation. In this presentation, our studies to improve TPLSM by utilizing some of novel optical technologies are introduced as follows.

1) In order to enhance penetration depth of the TPLSM, we applied a novel gain switched semi-conductor laser light source generating high-peak-power and stable light pulses. The developed system enabled the observation of the hippocampal CA1 cells with fine dendrite structure and granule cells in dentate gyrus in a living mouse [1]. Furthermore, by utilizing novel Ca^{2+} indicator, XCaMP-R, spontaneous firing of hippocampal CA1 cells in a mouse were visualized with a video-rate temporal resolution [2]. On the other hand, we recently adopted other technologies such as adaptive optics [3] or nano-sheet based observation methods [4] for deeper and sharper in vivo imaging.

2) The spatial resolution of TPLSM has been improved by applying super resolution microscopic techniques. Among them, we developed a compact stimulated emission depletion (STED) TPLSM that utilized electrically controllable components, transimissive liquid crystal devises and laser diode-based light sources [5]. The spatial resolution of the developed system was up to five times higher than conventional TPLSM systems [6].

3) A confocal spinning disk scanning unit and high-peak-power laser light sources were utilized to achieve high-speed TPLSM imaging. The developed system achieved larger field of view than using a mode-locked titanium-sapphire laser light source, superior spatial resolution than the TPLSM using non-descanned detector and superior penetration depth than single-point scanning single-photon excitation-based confocal microscopy [7], and has been used for several biological applications [8].

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Investigation of the gating, inactivation and blockage of a calcium gated ion channels using molecular dynamics simulations and cryo-EM

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[a]*School of Science, RMIT University, Melbourne, Australia*
[b]*Department of Anesthesiology, Weill Cornell Medicine, New York*
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Calcium gated potassium channels are widely distributed in the body where they regulate synaptic transmission, cell motility, gene transcription, muscle contraction and exocytosis. To transfer signals these channels cycles though open, inactivated and closed states. For some time there has existed uncertainty as to where the gate in these channels is located. Quaternary ammonium ions, which are known pore blockers of calcium gated potassium channels, have been shown to bind both to open and closed channels, suggesting that closed channels do not exhibit a narrow constriction in their intracellular-facing pores, but instead must gate/activate within their selectivity filters. However, new structures of the calcium-gated MthK channel, from *Methanothermobacter thermautotrophicus,* reveal that its intracellular-facing pore narrows to form a tight constriction in the closed state, apparently preventing blocker entrance into the pore. We have used cryo-electron microscopy and molecular dynamics simulations to describe the molecular gating mechanism, including distinct open and closed states, conformational changes related to channel gating, as well as the never-before-seen process of ball-and-chain inactivation. We have identified fenestrations in the transmembrane domain, not present in the open channel, which may facilitate access for molecules into the closed pore. We used 2-dimensional Umbrella Sampling simulations to show that quaternary ammonium ions can access the closed pore via these fenestrations with high rates, resolving previous uncertainties.

Vibrational spectroscopic study of G protein-coupled receptor

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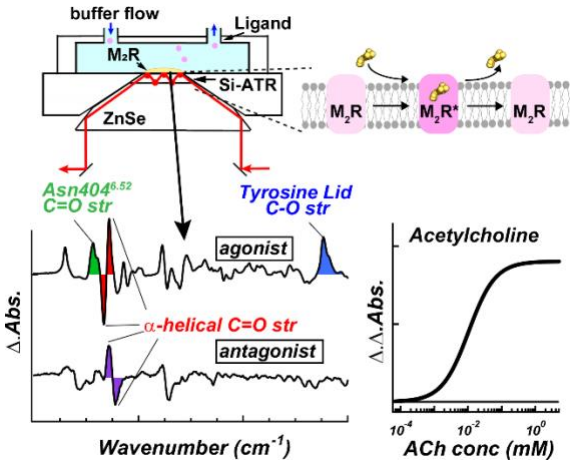
G protein-coupled receptor (GPCR) signalling utilizes an allosteric coupling between the extracellular facing ligand-binding pocket and the cytoplasmic domain of the receptor that selectively interacts with the signalling transducer such as G-proteins, β-arrestins and various other effectors. This allosteric effect enables one site of the receptor to regulate the function of another spatially distinct region. Therefore, it is important to understand the molecular mechanisms behind ligand-induced changes in receptor conformation for the development of GPCR-based drugs. Spectroscopy studies including EPR and NMR have recently demonstrated the dynamic behavior of GPCRs during ligand binding.

IR spectroscopy is also an excellent method for analyzing structural changes related to function of membrane protein, in particular photoreceptors such as cone opsins which belong to visual GPCRs [1, 2]. However, IR spectroscopy has not been fully exploited to study non-visual GPCRs. Recently, we have attempted to use Attenuated Total Reflection (ATR)-Fourier Transform IR (FTIR) spectroscopy with combining a two-liquid exchange system to study the conformational changes in muscarinic acetylcholine receptor (M_2R) that are induced by ligand binding. And, we have successfully measured the natural ligand acetylcholine (ACh) as an agonist binding-induced and atropine (Atro) as an antagonist binding-induced difference spectra, respectively [3]. Furthermore, by tracking ligand concentration dependence and binding/dissociating in real time, we successfully obtained physicochemical parameters such as ligand binding constants and rate constants simultaneously.

In addition, we also performed systematic ligand binding-induced difference ATR-FTIR spectroscopy on ligands with four different efficacies (agonist, partial agonist, antagonist, and inverse agonist). By monitoring the C=O stretch of amide-I band, distinct conformational changes were observed among the agonist, partial agonist, and antagonist, from which the degree of vibrational band change correlated with the functional results of G-protein activity in the cells. These results strongly indicate that the amide-I band can serve as an infrared probe to distinguish the ligand efficacy of M_2R.



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Discovery of an unusual activation mechanism for the TipA multidrug-resistance transcriptional regulator

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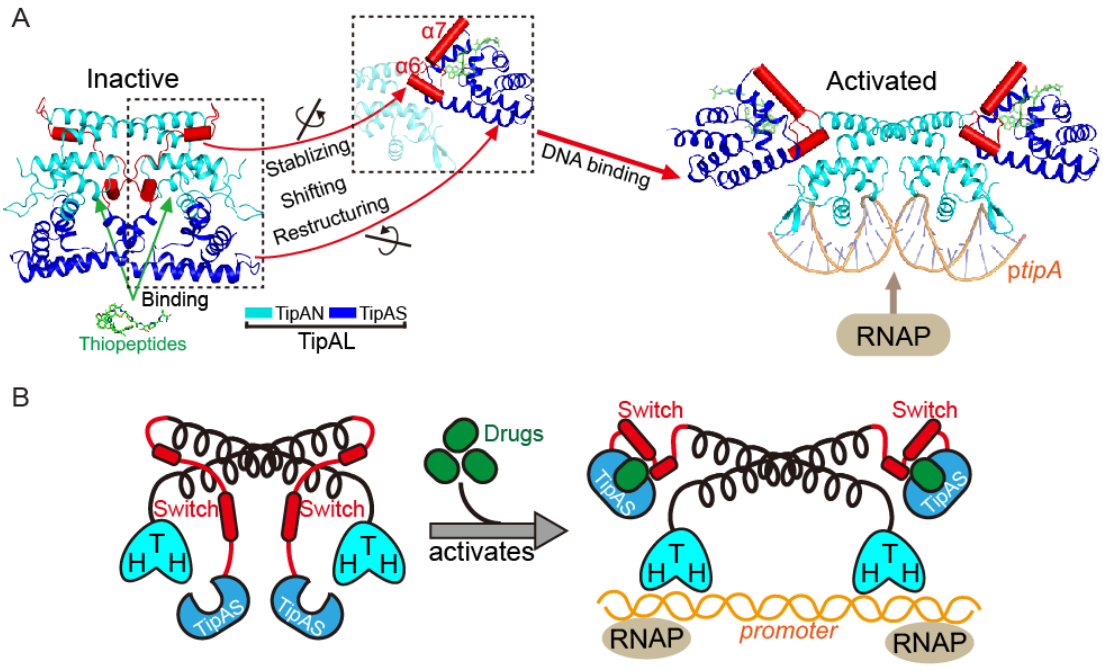
Investigations of bacterial resistance strategies can aid in the development of new antimicrobial drugs as a countermeasure to the increasing worldwide prevalence of bacterial antibiotic resistance. One such strategy involves the TipA class of transcription factors, which constitute minimal autoregulated multidrug resistance (MDR) systems against diverse antibiotics. However, we have insufficient information regarding how antibiotic binding induces transcriptional activation to design molecules that could interfere with this process. To learn more, we determined the crystal structure of SkgA from *Caulobacter crescentus* as a representative TipA protein. We identified an unexpected spatial orientation and location of the antibiotic binding TipAS effector domain in the apo state. We observed that the α6-α7 region of the TipAS domain, which is canonically responsible for forming the lid of antibiotic binding cleft to tightly enclose the bound antibiotic, is involved in the dimeric interface and stabilized via interaction with the DNA-binding domain in the apo state. Further structural and biochemical analyses demonstrated that the unliganded TipAS domain sterically hinders promoter DNA binding, but undergoes a remarkable conformational shift upon antibiotic binding to release this autoinhibition via a switch of its α6-α7 region. Hence, the promoters for MDR genes including *tipA* and RNA polymerases become available for transcription, enabling efficient antibiotic resistance. These insights into the molecular mechanism of activation of TipA proteins advance our understanding of TipA proteins as well as bacterial MDR systems, and may provide important clues to block bacterial resistance.



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Interactions of cholesterol-tagged DNA and lipid bilayer membranes

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Liposomes are aqueous vesicles enclosed by lipid bilayer membranes, used in research as simple, synthetic analogues of cell membranes as well as in medicine as nanoscale capsules for therapeutic compounds. Interest in modifying the shape, porosity and reactivity of liposomes has led to the development of functional, membrane-binding nanostructures made from DNA. The basis of DNA membrane-binding activity is the modification of DNA strands with hydrophobic or amphipathic chemical groups such as cholesterol. However, the interactions of cholesterol-modified DNA in solution and at membrane surfaces can be affected by factors such as DNA-DNA aggregation, buffer and lipid composition, and the biophysical properties of lipid bilayers [1].

We used fluorescence microscopy to characterise how buffer composition (monovalent and divalent ion concentration) and the configuration of cholesterol-tagged oligonucleotides affected DNA-lipid binding in simple nanostructures. We investigated how variations in the design and cholesterol modification of a classic single-layer DNA origami tile [2] influences membrane binding. Our results provide guidelines for maximising the binding yield of DNA to lipid bilayers and offer insight into how cholesterol-modified DNA interacts with lipid bilayers on a molecular level [3].

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Session 4: Australian Society for Biophysics NZ section

13:00-15:10

In vivo calcium imaging in mouse models of disease

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Calcium imaging is now a widely used technique to study the function of neural circuits in health and disease. This can now be done at a high level of detail, using either benchtop or miniaturised microscopes (miniscopes). Benchtop microscopes require head fixation to secure the animal, though the animal can walk on a treadmill during imaging. These microscopes allow for multiphoton imaging, which offers high resolution and is especially advantageous for light-scattering tissue. We are currently customising our two-photon microscope for imaging in conscious, moving rodents. Miniscopes are single-photon, lightweight (<3 grams) microscopes that are mounted onto the head of rodents, allowing cellular activity to be imaged in freely moving animals. We are currently utilising miniscopes to record neuronal activity in the hippocampus, in a mouse model of Alzheimer's Disease.

High-content super-resolution microscopy

David Baddeley, University of Auckland

Super-resolution microscopy methods such as PALM and STORM allow resolution on the scale of a few 10s or nm using visible light, and are becoming increasingly mainstream. The typical PALM/STORM experiment, however, is still time consuming, labour intensive, and looks only at a very small number of cells. As a result, the interpretation of results obtained is often also rather qualitative. I will discuss our implementation of a high–throughput super-resolution platform capable of automatically imaging of 10,000 cells in day, and our approach for handling the significant (~70TB/day) volume of data produced. I will also touch on our efforts to improve quality, ease of use, and to extract as much information out of data as possible. The combination of super-resolution and high-throughput allows us to image large populations of cells on molecular length scales, and to compare the results to population based methods such as genomics

The role of cardiac ryanodine receptor clusters in calcium leak and arrhythmia

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The ryanodine receptor (RyR) is a calcium-sensitive calcium channel localised to the sarcoplasmic reticulum (SR) of the myocyte, where it is arranged into clusters and releases calcium from the SR. This calcium release is essential for cardiac function; however abnormal RyR activity can lead to calcium leak and the development of arrhythmias, including atrial fibrillation (AF). When RyR clusters are localised within a sufficiently small distance of each other, there is the potential for co-activation of neighbouring clusters, such that clusters can form a functional calcium release unit (CRU). Previous studies reveal that remodelling of RyR clusters and CRUs occurs in pre-clinical models of AF and heart failure, and is associated with increased calcium leak and enhanced arrhythmogenesis. Whether such remodelling also occurs in diseased human cardiac tissue remains unexamined. Therefore, using super resolution imaging (dSTORM), we analysed the nanoscale organisation of RyR clusters in atrial myocytes from patients with paroxysmal and persistent AF. Persistent AF patients demonstrated significant atrial dilation and myocyte hypertrophy, confirming the presence of pathological remodelling in these patients compared to non-AF. Interestingly, RyR clustering properties were remarkably unaltered in the AF patients. No differences in individual cluster or CRU organisation parameters were identified in either the paroxysmal or persistent AF patients compared to non-AF. These findings indicate that unlike pre-clinical models of cardiac disease, RyR cluster remodelling is not a contributing mechanism to the altered channel function and arrhythmogenic activity observed in AF patients.

Fibrosis and impaired Ca^{2+} signalling in heart failure

David Crossman[a]
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Heart failure (HF) is a devastating condition in which the heart is unable to produce enough blood flow or cardiac output to supply the body's energy requirements. Two major pathological features of the failing heart are (1) fibrosis and (2) aberrant intracellular Ca^{2+} handling. In the HF literature, these are considered separate features. However, our recent publication indicates that fibrotic disposition of nanometre-sized collagen fibrils may directly cause diminished Ca^{2+} signalling in HF [1]. In HF with reduced ejection fraction pathological remodelling of the transverse (t)-tubules is thought to drive dyssynchronous Ca^{2+} release and reduced force of contraction. We have identified with super-resolution microscopy that remodelled t-tubules in the failing heart have increased amounts of collagen within their lumen indicating that the process of fibrosis can directly disrupt t-tubule structure and the associated Ca^{2+} signalling apparatus with the cardiac myocyte.

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N-glycans – the sticky ends for shear force activation of epithelial Na^+ channel (ENaC)

Jan-Peter Baldin[a], Daniel Barth[a], Fenja Knoepp[c] and Martin Fronius[a,b]
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[c] *Cardio-Pulmonary Institute, University Giessen, Giessen, Germany*

The epithelial Na^+ channel (ENaC) is widely expressed throughout the human body and important for Na^+ and water homeostasis and blood pressure regulation. Canonical ENaC formed by α, β and γ subunits increase its open probability when exposed to shear force. Recent evidence identified specific N-glycans of the α ENaC subunit and the extracellular matrix (ECM) to be important for ENaCs ability to respond to shear force [1], indicating that ENaC is gated by the force from filament principle. Aim of this study was to reveal whether the activity of N-glycans and the extracellular matrix is interdependent and whether N-glycans of β and γ ENaC have similar roles shear force activation of ENaC as identified in α ENaC.

Combinations of ENaC subunits were injected in *Xenopus* oocytes and used for two electrode voltage clamp experiments to assess ENaC currents. Shear force was applied by exposing oocytes to a fluid stream through the perfusion of a custom-made chamber. The role of N-glycans was addressed by the introduction of glycosylation motifs, or by disruption of the glycosylation motifs by site-directed mutagenesis. The role of the extracellular matrix was addressed by the use of hyaluronidase, an enzyme that degrades hyaluronic acid of ECMs. Shear force responses of cells expressing mutated channels or degraded ECM were compared with those expressing wild type (wt) channels and having an intact ECM.

The introduction of glycosylation motifs into δ ENaC did results in channels that provided a stronger response when exposed to shear force in comparison to the wt channel. This increased response was abolished in cells that were treated with hyaluronidase. In contrast, removal of the N-glycans of β or γ ENaC did not impair the ability of the channels to respond to shear force. With one exception - the removal of N-glycans at position 378 of β ENaC did yield a channel that was more responsive to shear force compared with the wt channel.

In summary, certain N-glycans of ENaC play an important, but different role for the channels ability to respond to shear force. While some N-glycans seem to be needed to enable mechanical activation (e.g. by providing a connection to the ECM), one N-glycan of β ENaC seems to impair shear force activation of ENaC, as its removal made the channel more susceptible to shear force. It might be speculated that in both cases N-glycans act as sticky ends allowing physical interactions between molecules and are important for mechanical activation of ion channels that are gated by the force from filament principle.

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Bee flight muscle and their paradoxes.

Tony Hickey,
School of Biological Sciences, University of Auckland

Bumblebee flight was once a paradox, as they appear to defy gravity. The high wing speeds of bees permit flight, even at air densities lower than Mount Everest's summit (~9000m). Yet flight muscles (FMs), which account for 30% of mass lift nearly double a bee's mass. As bee wings beat at ~230Hz (hummingbirds ~90Hz), FMs consume O_2 at the fastest rates recorded with mitochondria (*mt*) packed to the upper limits of power production (~40% volume).

Yet while flight is metabolically expensive, paradoxically bee FM *mt* is likely inefficient, as they waste significant energy as heat, they release 30x more reactive oxygen species (ROS) than rat heart *mt* and oddly there are apparent issues with ATP diffusion. FM *mt* are stacked twice as far apart than *mt* in vertebrate muscles, increasing ATP diffusion distance (Figure), and the creatine kinase shuttle (CK-S) appears to be absent in bee FM. Depression of the CK-S in mammals coincides with heart failure heart failure and increased mt ROS production Moreover, FMs have little LDH activity. While the CK-S and LDH buffer localised ATP demands, bees likely compensate for low LDH using the glycerol 3-phosphate (G3P) shuttle, we found the G3P pathway increases ROS.

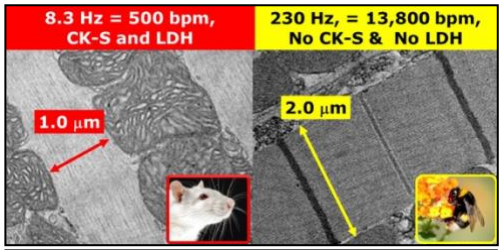

Fig1. Despite ~30x faster beat rates in the bee, rat heart muscle fibrils are half as thick as bee FM. This increases diffusion distances in bee FM, which lacks LDH and the CK-S.

So, how do bee FMs overcome ATP diffusion barriers, and how are ROS managed?

Session 5: Computational Biophysics

15:30-17:15

Initiation of Cardiac Arrhythmias by Heart Cells that Fail to Repolarise

Stewart Heitmann[a], Jamie I Vandenberg[a,b] and Adam P Hill[a,b]
[a] *Victor Chang Cardiac Research Institute, Darlinghurst, NSW 2010, Australia.*
[b] *University of NSW, Kensington, NSW 2010, Australia.*

Cardiac arrhythmias can be initiated by heart cells that spontaneously depolarize after a normal heartbeat. Those after-depolarizations induce re-entrant activity in the heart tissue which ultimately disrupts normal heart function. Here we propose an alternative account of arrhythmogenesis in which arrhythmias are triggered autonomously by cardiac cells that fail to repolarize after a normal heartbeat. We investigated the proposal by representing the heart as an excitable medium of FitzHugh-Nagumo cells where a proportion of cells were capable of remaining depolarised indefinitely. Such cells are said to have bistable membrane dynamics. We found that the medium could tolerate a surprisingly large number of bistable cells and still support normal rhythmic activity. Yet there is a critical limit beyond which the medium is chronically arrhythmogenic. Numerical analysis revealed that the critical threshold for arrhythmogenesis depends on both the strength of the coupling between cells and the extent to which the abnormal cells resist repolarization. Arrhythmogenesis was also found to emerge preferentially at tissue boundaries where cells naturally have fewer neighbours to influence their behaviour (Figure 1). That finding may explain why atrial fibrillation typically originates from the site where the pulmonary vein attaches to the wall of the heart.

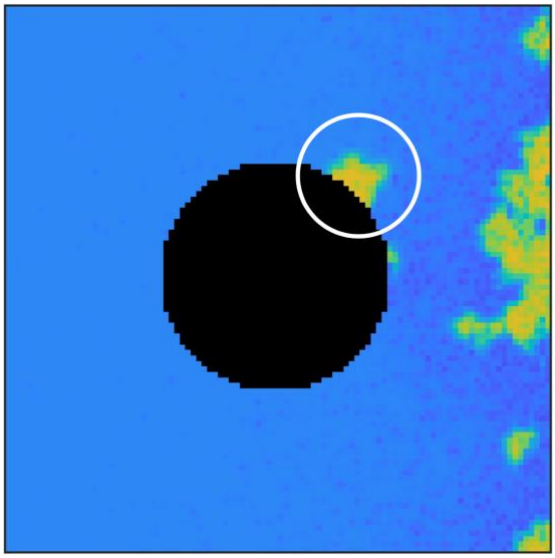

Figure 1: Ectopic activity (white circle) emerging from a tissue boundary after a normal heartbeat. The square domain represents heart tissue. The black disc represents a vasculature opening where cells are absent. Colour indicates the membrane potential of the cells.

The potential role of the physical and chemical arrangement of erythrocytes membrane molecules on its mechanical behavior: A molecular dynamics simulation

Hossein Borazjani, Jafar Rouzegar and Omid Bavi
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A better understanding of the cell membrane of living species requires the study of individual lipid and protein components. effective physical and chemical properties such as geometry and chemical composition of lipid molecules, packing density (area per lipid), acyl chain length (membrane thickness) unsaturated bonds in acyl chain, and charge density of headgroup atoms of membrane describe the mechanical properties of lipid bilayers [1, 2]. The mechanical behavior of biological membranes is one the most essential characteristics for practical application in three areas: I) Treatment (differentiation between healthy and cancerous cells), II) Liposomal smart drug delivery systems [3], and III) Energy and environment (Design of desalination systems with biological membranes and ion channels [4]). In this research, using all-atom molecular dynamics simulation, the effect of unsaturated hydrocarbon chains and the percentage of lipid molecules on the mechanical properties of erythrocyte cell membrane have been studied. The values obtained for the lipid surface area and the area compressibility modulus (KA) of the erythrocyte cell membrane indicate that the strength of this membrane is comparable to that of a strong lipid such as Ester DPhPC bilayer (approximately 265 mN/m) [5]. Since the erythrocyte membrane is constantly exposed to the mechanical shear forces of the blood fluid, it is expected to be a suitable option for use in applications such as membrane desalination systems.

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Theoretical studies on the selectivity of inhibitors on FGFRs by molecular dynamic simulations and free energy calculations

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The essential and necessity of drug discovery in the case of target evaluation and lead to optimization, which is so regular, is the selectivity of drug candidates. Whereas poor selectivity of drug candidates may face problems such as leading to toxicity and side effects accounting for more than 50% failure rate [1]. Absolutely, one of the biggest barriers, which is so important to understand target biology and mitigating off-target toxicity, in the early steps of the process related to inhibitor discovery is selective inhibition. Whenever a lead compound should be optimized to be selective, it is logical to clarify the mechanism of selectivity. Therefore, in silico method will be used by doing some analysis regarding the pocket similarities of several proteins and checking effective residues. Therefore, all efforts made will help medicinal chemist to recognize potential secondary pharmacology, describe the relationship in association with the structure-activity, and finally get control of selectivity issues [2-4].

Fibroblast growth factor receptors (FGFRs) are natural molecules with a protein or steroid structure involved in angiogenesis, wound healing, and embryonic development. These factors are involved in the growth and differentiation of many cells and are a type of cytokine [5]. Development of new selective inhibitors against these receptors family requires the understanding of the binding mechanism of potent inhibitors against each FGFRs. To aim this purpose, we calculated the ligand binding energy using molecular dynamics simulations on the basis of the MM-PBSA/GBSA method [6] and its analysis was carried out to examine the molecular interaction effectiveness of four selected ligands with the active site of each FGFRs (FGFR1 to 4).

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The Influence of the Choice of Force Field on the Characterisation of the Monomeric Form of Rat and Human Islet Amyloid Polypeptide

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Human islet amyloid polypeptide (hIAPP) is a naturally occurring, intrinsically disordered protein (IDP) whose abnormal aggregation into toxic soluble oligomers and insoluble amyloid fibrils is a pathological feature in type-2 diabetes. Rat IAPP (rIAPP) differs from hIAPP by only six amino acids and yet does not have a high tendency to aggregate or form fibrils. The structures of the monomeric forms of IAPP are thus difficult to characterise due to their intrinsically disordered nature. Molecular dynamics (MD) simulations can provide detailed characterisation of the monomeric forms of rIAPP and hIAPP in near-physiological conditions. In this work the conformational landscapes of rIAPP and hIAPP were predicted as a function of secondary structure content using well-tempered metadynamics with multiple walkers. Several commonly used biomolecular force fields in combination with multiple water models were tested. The predicted conformational preferences of both rIAPP and hIAPP are typical of IDPs, showing both slight alpha-helical and beta-sheet content but with the lowest free energy states reflecting dominant random coil structures. Predicted NMR Cα chemical shift show different preferences with each force field towards certain conformations. Comparisons of secondary structure content demonstrate the importance of the choice of force field when characterising IDPs.

Characterization of amyloid β peptide structural ensemble

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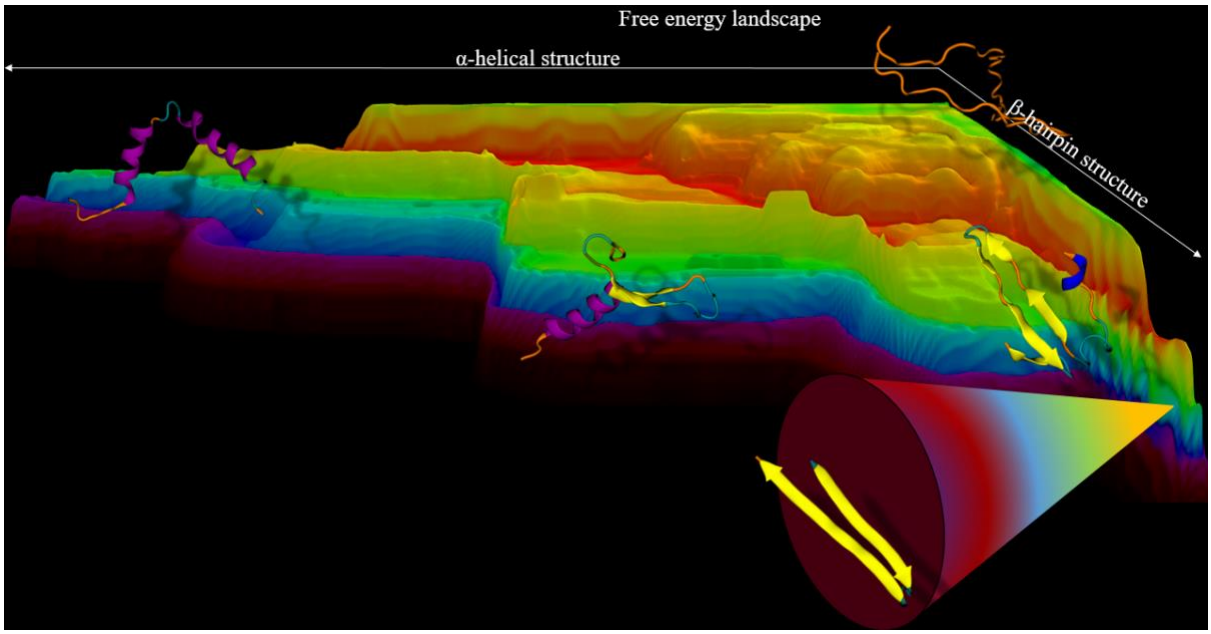
Alzheimer's disease (AD) is characterised by the formation of amyloidogenic protein plaques, primarily made up of aggregated amyloid β42 (Aβ42) in the brain. The fibrillar forms of Aβ42 aggregates exhibit well-defined, beta-sheet structures at the molecular level and are insoluble and less toxic than the soluble, disordered oligomeric forms. However, the mechanism of interaction of monomers and the subsequent process of oligomerisation is not well understood. The intrinsically disordered nature of Aβ42 adds to the challenges in determining the structural properties of monomers. We have used enhanced molecular dynamics simulations to explore the conformational landscape of monomeric Aβ42, focusing on conformations that might be of relevance for the early stages of oligomerisation. After analysing the simulation, we found out range of meta-stable structures of AB42 monomer that might play role in initial aggregation of dimers and trimers.



A Molecular Docking and Molecular Dynamics Study on Prostate-Specific Membrane Antigen (PSMA) to design effective small molecules as inhibitors

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Prostate Cancer is one of the most common cancers and the second deadliest cancer in men that can be controlled with antibody therapy [1]. Given that, in this type of disease, a significant increase in prostate-specific antigen and prostate-specific membrane antigen (PSMA) occurs, the primary principle in vaccination against this type of cancer is the use of these antigens [2]. Current research, through pharmacodynamic experiments on a library of 13 million natural compounds in the ZINC database, sought to identify compounds that can suppress the PSMA protein. In the early stages, the compounds selected as inhibitors for further research were scanned by the library with pharmacophore and ADMET analysis. The ADMET is typically used in silica models for fast and initial screening of ADMET properties before further evaluating compounds in vitro [3]. In the next step, using LeDock to study molecular docking [4], fifteen ligands with the best state related to the binding results were selected and to evaluate the stability of the structure related to the ligand-protein complex, molecular dynamics simulation by AMBER 18 software package and a parallel version of SANDER was performed. Due to the fact that this protein contains zinc ions and its mechanism is completely dependent on the presence of zinc ions, all simulation steps are performed without restriction, so that in all stages and analysis, natural conditions prevail. After performing the steps related to virtual screening, docking, molecular dynamic simulation and selecting the best structures as inhibitors, the MMPBSA/MMGBSA method [5] was used to calculates binding free energy.; Using decomposition and hydrogen bond analyses, effective interactions between selected ligands and important residues in the S1 and S1´ pockets related to the protein active site have been discussed. With the relevant analyses, the importance of the presence of zinc ions in the active site, which has an undeniable effect on the mechanism of selected ligands, has also been discussed. Finally, the proposed structures for synthesis in laboratory conditions were presented as a suggestive effective inhibitor of small molecules as ligands.

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How does calcium make the heart grow?

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Calcium (Ca2+) plays a central role in mediating both contractile function and hypertrophic signaling in ventricular cardiomyocytes. L-type Ca2+ channels trigger release of Ca2+ from ryanodine receptors for cellular contraction, whereas signaling downstream of G-protein-coupled receptors stimulates Ca2+ release via inositol 1,4,5-trisphosphate receptors (IP3Rs), engaging hypertrophic signaling pathways. Modulation of the amplitude, duration, and duty cycle of the cytosolic Ca2+ contraction signal and spatial localization have all been proposed to encode this hypertrophic signal. In this talk I will present biophysics-based computational models that provide new insights about how IP3Rs induced Ca2+ may effect hypertrophic gene transcription.

Day 3: 4th December 2020

Session 6: Plenary/Prize session

09:00-11:00

Force-from-Lipids Gating in Mechanosensitive Channels

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Prokaryotic mechanosensitive (MS) channels open by sensing the physical state of the membrane. As such, lipid-protein interactions represent the defining molecular process underlying mechanotransduction. Here, we describe cryo-electron microscopy (cryo-EM) structures of the *E. coli* small-conductance mechanosensitive channel (MscS) in nanodiscs (ND). They reveal a novel membrane-anchoring fold that plays a significant role in channel activation and establish a new location for the lipid bilayer, shifted ~14 Å from previous consensus placements. Two types of lipid densities are explicitly observed. A phospholipid that “hooks” the top of each TM2-TM3 hairpin and likely plays a role in force sensing, and a bundle of acyl chains occluding the permeation path above the L105 cuff. These observations reshape our understanding of force-from-lipids gating in MscS and highlight the key role of allosteric interactions between TM segments and phospholipids bound to key dynamic components of the channel.

Locating antimicrobial peptides in model membranes: comparison of the action of carpet and pore forming peptides via neutron reflectometry

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Antimicrobial peptides (AMPs) are essential components of host defence against infections, serving as the first line of innate immunity against bacterial and fungal infections in a wide range of organism. They may offer a solution for the global health threat of antibiotic resistant bacteria [1]. Despite the wide-ranging studies on AMPs, in most cases the molecular process that leads to membrane disruption is not well understood, due to the difficulty of gathering direct evidence for their mechanism of action. Melittin (from bee venom) and aurein 1.2 (secreted by Australian tree frog Litoria aurea) are two well-known AMPs with substantive studies on their mechanism of action, demonstrating transmembrane and surface acting mechanism, respectively [2,3]. Although the mechanism of action of these two AMPs with model membranes have been widely studied, there are still uncertainties about their orientation throughout the interaction. In this study neutron reflectometry measurement was used to determine and compare the position of the peptides in the DMPC (dimyristoylphosphatidylcholine) membrane at different stages of disruption due to the highly concentration-dependent mechanism.

The analysis of neutron reflectometry measurements reveals the peptide position in the membrane at different stages of each specific model mechanism. As expected, it was shown that at low concentrations both peptides are mainly located in the top leaflet bound to the head group region regardless of the mechanism. The neutron reflectometry analysis of aurein 1.2 shows that by increasing the concentration, the swelling of top leaflet and potential membrane blistering is detected, consistent with the well-accepted surface tension-driven carpet-like mechanism. In the case of melittin it was revealed that by increasing the concentration, initially the peptide penetrates the tail region of the upper membrane leaflet, and further increase in concentration leads the peptide to reach the bottom leaflet. These findings confirm the hypothetical mechanism of each peptide presented before based on quartz crystal microbalance studies [4,5].

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Understanding the biophysical properties of the plasma membrane through computational modelling

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Research School of Chemistry, College of Science, The Australian National University, Canberra, ACT, 2601, Australia

Cell membranes contain incredible diversity in the chemical structures of their individual lipid species and the ratios in which these lipids are combined to make membranes. Nevertheless, our current understanding of how each of these components affects the properties of the cell membrane remains elusive, in part due to the difficulties in studying the dynamics of membranes at high spatiotemporal resolution. Coarse-grained molecular dynamics simulations provide unprecedented insights into the behaviour of membranes. In this talk I will highlight how changes to the composition of the plasma membrane that occur in different tissues effect the biophysical properties of the membrane. Additionally, I will show how approximating the membrane composition through including fewer lipid species can significantly alter these membrane properties (Figure 1). Overall, these results show how subtle chemical changes can affect the properties of the membrane and highlight the lipid species that give plasma membranes their unique biophysical properties.

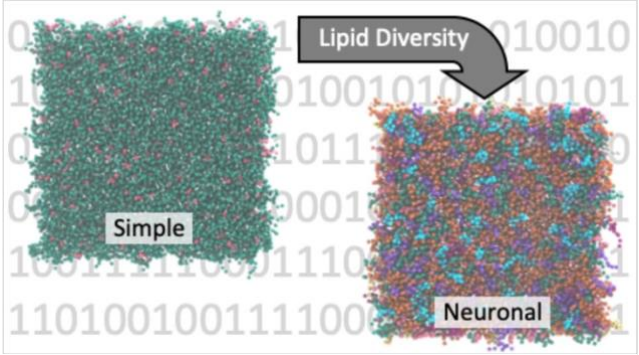

Figure 1. Changes to neuronal membrane composition investigated through 8 increasingly complex membranes.

Session 7a: General Biophysics
12:30-14:30

An acoustic platform for single-cell, high-throughput measurements of the viscoelastic properties of cells

Valentin Romanov[a]*, Giulia Silvani[a]*, Huiyu Zhu[c], Charles D Cox[a,b], Boris Martinac[a,b]
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[b] *St Vincent's clinical School, University of New South Wales, Sydney, NSW, Australia*
[c] *University of Technology Sydney, Ultimo, Sydney, NSW, Australia*
**These authors contributed equally*

Background

Numerous reports have been published looking at the effect of either cytoskeletal or plasma membrane modifications on the viscoelastic properties of cells [1]. For instance, optical tweezers have been used to show cell stiffness and fluidity modulation based on enrichment or depletion of cholesterol [2]. Changes to protein expression, whether through over-expression of lamin A/C, an intermediate filament protein localized in the nuclear envelope [3] or the knockout of vinculin, a protein of the focal adhesion complex [4], have been shown to have a measurable effect on the viscoelastic properties of cells. While a number of techniques have been developed to capture the effects of protein expression on cell viscoelasticity, high throughput techniques, capable of measuring single-cells, are lacking.

Methods

Human Embryonic Kidney 293T (HEK293T) cells over expressing the transmembrane mechanosensitive ion channel, Piezo1, were used as model adherent cells, developed from Piezo1 knockout cells. Viscoelastic properties of cells as a function of temperature, pharmacological treatments and varying expression levels of Piezo1 were characterized by Acoustic Force Spectroscopy (AFS). AFS, developed by Lumicks B.V. is an acoustic platform, utilizing a transparent piezo membrane element to acoustically drive particles within a microfluidic environment.

Results

We show that the AFS can be used to characterize the viscoelastic properties of HEK293T in high throughput fashion, with an experimental throughput of about 80 to 180 cells per hour. In doing so, we present a new tool for probing the material properties of adherent cells. We validate the AFS by demonstrating that it can capture changes in cell viscoelasticity based on rapidly changing environmental temperatures, ranging from 21 °C to 37 °C. In addition, we show quantitative changes to the cytoskeleton upon the addition of either Cytochalasin D or Colchicine. Finally, we demonstrate that the protocol developed here, for the AFS, can be used to measure changes in cell viscoelasticity based upon the expression of Piezo1.

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Probing mechanosensitivity at the cell-substrate interface

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Mechanical force plays a vital role in our life. They are the primary reason underlying our sensation of touch, pain, blood pressure, balance and hearing. The fastest transducer of these mechanical forces in our body are a set of ion channels that are embedded in the cell membrane and classified as mechanically-gated (MG) channels (Cox et al, 2019 Cell Reports). The presence of distinct MG channels within one cell may enable the cells to integrate different types of mechanical inputs. A fascinating open question in the study of MG ion channels relates to how exactly an applied force can lead to channel gating. Addressing this question is additionally hampered by the challenges in understanding how distinct approaches to deform cells and membranes impact such parameters such as membrane stretch/bending and/or cortical tensions. As such, we have used a blend of theoretical (e.g. finite element) and experimental (e.g., pillar array, high-speed pressure clamp and Ca^{2+} imaging) techniques to probe how mechanical properties of the substrate, such as stiffness and roughness, modulate the force sensitivity of the well-characterised mammalian channel, PIEZO1 (Bavi, et al., 2019 ACS nano). Moreover, we showed how different channels respond differentially to changes in the properties of the cellular substrate (Piezo1 vs K_2P channels), reinforcing the need to understand how forces propagate through biological structures. As such, our approach to studying MG channel function, combined with the techniques described earlier provide a powerful insight into the diversity of force sensing via MG ion channels.

Deciphering the assembly and signalling mechanisms of the unique BAFF 60-mer through the TACI cytokine receptor

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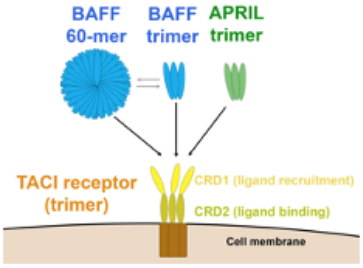
[b] *Department of Microbiology and Immunology, Peter Doherty Institute, Victoria, Australia.*

[c] *QIMR Berghofer Medical Research Institute, Queensland, Australia.*

[d] *Australian Cancer Research Foundation Rational Drug Discovery Centre, St. Vincent's Institute of Medical Research, Victoria, Australia.*

The transmembrane activator and cyclophilin ligand interactor (TACI) receptor regulates the adaptive immune system and is involved in B-cell biology. TACI has been validated as a target for the treatment of chronic lymphocytic leukaemia. However, how TACI signals remains elusive. The TACI receptor has two extracellular domains, with the membrane-proximal domain binding to two cytokines, B-cell activating factor (BAFF) and A proliferation-inducing ligand (APRIL).



It has been previously demonstrated that a trimer of APRIL binds to a trimer of the TACI receptor on the cell surface, activating signalling [1]. On the other hand, BAFF has been reported to exist both as a trimer and a 60-mer [2, 3] . This is the only known example of a cytokine adopting a 60-meric conformation, reminiscent of a viral capsid. The physiological and pathological relevances of the two oligomeric states of BAFF remains unknown, as well as the assembly/disassembly mechanism by which BAFF switches between its two forms.

We have used a multi-pronged approach including mutagenesis, biophysical assays, X-ray crystallography and cryo-electron microscopy to explore BAFF biology . For the first time, we have identified environmental conditions and mutations that cause disassembly of the BAFF 60-mer into a trimer. These studies have revealed a novel intermediate disassembly step consisting of a pentamer of trimers that has been captured by cryo-electron microscopy. Computational studies have led to the design of a peptide that prevents the BAFF trimers from assembling into the 60-mer. The binding affinity of this peptide to BAFF was determined using surface plasmon resonance and preliminary co-crystals were obtained. We have also successfully purified the ligand-binding domain of the TACI receptor to investigate its binding to the two forms of BAFF using biophysical assays, crystallography and electron microscopy. These studies provide a step-by-step mechanism for the activation of the TACI receptor by BAFF.

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Ionic basis of human Neurogenin-2 induced sensory neuron excitability

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Sensory perception is fundamental to everyday life, yet the understanding of human sensory physiology at the molecular level is hindered by constraints on tissue availability. Emerging strategies to study and characterize peripheral neuropathies, *in vitro*, involve the use of human pluripotent stem cells (hPSCs) differentiated into dorsal root ganglion (DRG) sensory neurons.
The currently available protocols efficiently yield sensory-like transcriptional signatures, however the resulting excitability profiles and functional analysis of the underlying conductances has remained underexplored in these sensory neuron.

An optimised protocol was developed combining exogenous expression of Neurogenin-2 (NGN2) and small molecule inhibitors to efficiently direct hPSC differentiation towards functionally mature neurons ($_{NGN2}$iSNs) expressing the sensory markers BRN3A, ISLET1, TRKA, TRKB and TRKC. The functional characterization of the resulting $_{NGN2}$iSN excitability profiles evidenced various modalities of action potential firing supported by robust voltage-gated sodium, potassium and calcium conductances. An in depth analysis of the molecular basis of $_{NGN2}$iSN excitability revealed functional expression of ion channels associated with excitability of primary afferent neurons including Nav1.7, Nav1.8, Kv1.2, Kv2.1, BK, Cav2.2, Cav2.1, Cav3.2, ASICs and HCN, which were also detected at the transcriptional and immunocytochemical levels. The excitability profile of human $_{NGN2}$iSNs is consistent with the generation of a functionally heterogeneous population of sensory neurons with passive and active membrane electrical properties akin to mature human sensory neurons.

A plethora of studies suggest that the pathophysiology of sensory neuropathies results from the dysfunction or dysregulation of neuronal ion channels hence recognized as *channelopathies*. The thorough molecular understanding of the conductances underpinning excitability is thus fundamental to the establishment of hPSC-derived sensory neuron cultures as a meaningful platform for human disease modelling and drug screening.

Repurposing the spider toxin Pn3a as a selective inhibitor within the T-type calcium channels

Jeffrey R. McArthur[a], Jierong Wen[b], Andrew Hung[b], David J. Adams[a] and Rocio K. Finol-Urdaneta[a]

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There is a large variety of ion channels expressed in peripheral nociceptive neurons that contribute to the transduction and/or propagation of physiological and pathophysiological stimuli. T-type calcium (Cav3), and sodium (Nav) channels play central roles in controlling excitability within the central and peripheral nervous system, and therefore constitute potential hyperexcitability disorder targets, including pain. Despite their differing functions and ion selectivity, these channels share similar architecture comprised of a pore region surrounded by four voltage-sensing domains (VSD), in a more or less four-fold symmetric arrangement. Such structural conservation prompted the question of what effects modulators of Nav channels, regarded as analgesic, would have at T-type calcium channels. T-type calcium channels are known off targets for Nav channel drugs as shown by examples like ProTxI/II, Kurtoxin and others, to date these compounds have only modest preferences between Cav3.1 and Cav3.2.

μ-Theraphotoxin-Pn3a (Pn3a), isolated from venom of the tarantula Pamphobeteus nigricolor, is an analgesic peptide shown previously to potently inhibit voltage-gated sodium channels. Pn3a is the first modulator of T-type calcium channels, with > 100-fold selective for Cav3.3 (IC50 960 nM) over both Cav3.1 and Cav3.2. Similar to its effects on Nav channels, Pn3a's modulation of Cav3.3 was via changes in channel gating, specifically resulting in a 10 mV depolarizing shift in channel activation, with no changes to steady-state inactivation. To elucidate Pn3a's binding site, we generated chimeras where each of the four VSD of Cav3.3 were structurally spliced onto the pore domain of Kv1.7. Only when Pn3a was applied to the Kv1.7/Cav3.3 DII chimera was there both observable inhibition of channel currents and a shift in the chimeric channel's activation profile. We were able to generate computational models of Pn3a's interactions with DII of Cav3.3 and compare binding energies across the 4 domains and across all T-type calcium channels to identify residues critical for Pn3a's binding and selectivity.

We have demonstrated cross-reactivity of Pn3a, a previously considered Nav channel "family selective" compound and identity a potential drug site on Cav3.3 which may allow new Cav3.3 selective therapeutics to be designed. Pn3a, as a 100-fold selective inhibitor of Cav3.3 will provide researchers with a tool to dissect and better study the role of this channel in physiology and pathophysiology. The structural conservation between Nav and Cav drug receptor sites suggests other potential useful compounds may be identified by screening previously described selective compounds against other ion channel families.

Asters, spirals and vortices revisited: ATP dependent structures in actomyosin

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Actomyosin, the canonical mixture of rod-like actin filaments and myosin motors, is both a central example of active matter and a key ingredient of the cell cortex [1]. It has been shown to play a crucial role in many cellular processes including locomotion, mechano-sensation and cytokinesis.

In a classic paper entitled “Vortices, Spirals and Asters”, the authors of [2] described how a generic active polar gel can give rise to the titular textures as steady states of the phenomenological hydrodynamic equations. However, although celebrated for the important ideas put forward, experimental verification of [2] has always been limited, at least in part, due to complex cellular signalling processes masking the underlying physics in living cells.

Now, in light of recent *in vitro* experiments with reconstituted actomyosin on a supported lipid bilayers [3,4], we re-examine the notion of such vortices, spirals and asters, and therefore some of the assumptions that underpin classical polar active gel theory as a model for actomyosin. In particular, we account for the microscopic kinetics of myosin mini-filaments, making use of a microscopic model to find scaling relations for our coarse-grain hydrodynamic parameters as a function of ATP concentration. We find two distinct regimes in our hydrodynamic equations, a contractile regime at low ATP and at high ATP a regime where myosin displays processive dynamics along slowly remodelling actin filaments, which is in agreement with recently published experiments [4].

We then proceed to compare these with iSCAT and TIRF experiments on a similar *in vitro* system to [3,4], but with longer actin filaments. A key new finding from these experiments is the formation of vortices/spiral structures at high ATP concentration which has not been seen before in *in vitro* systems. We find a quasi-steady state vortex/spiral solution in the high ATP regime of our equations which reproduces several key features of the vortices seen in experiment. We also find an aster steady state in the low ATP regime and compare this with contractile foci seen in the ATP depleted regime of the experiments. Finally we discuss where our theory breaks down and the need for new approaches in the hydrodynamic theory of actomyosin.

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Session 7b: Membranes and Membrane proteins
12:30-14:30

Structural basis of CXCR4 signalling illuminated by experimental and computational approaches

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Chemokines and their receptors are orchestrators of cell migration in humans. Dysregulation of the receptor-chemokine system leads to inflammation and cancer, and thus, both chemokines and receptors are highly sought therapeutic targets. One of the barriers for their therapeutic targeting is the limited understanding of the structural principles behind receptor-chemokine recognition and selectivity. The existing structures do not include CXC subfamily complexes and lack information about the receptor distal N-termini, despite the importance of the latter in signalling, regulation, and bias.

Here, we report the geometry of the complex between full-length CXCR4, a prototypical CXC receptor and driver of cancer metastasis, and its endogenous ligand CXCL12. By comprehensive disulfide crosslinking, we establish the existence and the structure of a novel interface between the CXCR4 distal N-terminus and CXCL12 β1-strand, while also recapitulating earlier findings from nuclear magnetic resonance, modelling and crystallography of homologous receptors. A crosslinking–informed high-resolution model of the CXCR4-CXCL12 complex pinpoints the interaction determinants and reveals the occupancy of the receptor major subpocket by the CXCL12 proximal N terminus. This newly found positioning of the chemokine proximal N-terminus provides a structural explanation of CXC receptor-chemokine selectivity against other subfamilies. Furthermore, key charge swap mutagenesis experiments provided additional evidence for pairwise interactions between oppositely charged residues in the receptor and chemokine, confirming the accuracy of the CXCR4-CXCL12 complex. Our findings challenge the traditional two-site understanding of receptor-chemokine recognition, suggest the possibility of new affinity and signalling determinants, and fill a critical void on the structural map of an important class of therapeutic targets. These results will aid the rational design of selective chemokine-receptor targeting small molecules and biologics with novel pharmacology.

Positive Feedback Loop Between E-cadherin and F-actin

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The cadherin-catenin-complex and the actomyosin cytoskeleton are two major players in adherens junctions. Their dynamics are reciprocally affected through biomechanical and chemical signals. Due to the complexity, various distribution patterns of cadherin have been observed without in-depth understanding of underlying mechanisms. In this study, we present a new computational model of intercellular junction maturation in a cell doublet. The model couples a 2D lattice-based model of cadherin dynamics with a continuum, reaction-diffusion model of the reorganizing actomyosin network through its regulation by Rho signaling at the intercellular junction. We demonstrate that local immobilization of cadherin induces cluster formation in a cis less dependent manner. As we know that actin affects cadherin dynamics, we also investigated how cadherin and actin regulate and cooperate with each other. By considering the force balance during intercellular adhesion maturation and the force sensitive property of the cadherin/F-actin linking molecules, we show that cortical tension applied on the contact rim can explain cadherin's and F-actin's spatial distribution on the cell-cell contact of the cell-doublet. Meanwhile, the positive feedback loop between cadherin and F-actin is necessary for maintenance of the ring. Different patterns of cadherin distribution can be observed in the model when this feedback loop is disturbed. We discuss these computational insights in light of available experimental observations on underlying mechanisms related to cadherin/F-actin binding and mechanical environment.

Characterization of slow and sub-diffusive behaviour in crowded protein solutions and discerning the underlying causal relations

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The environment inside cells is densely packed, termed macromolecular crowding, the extent of which varies throughout the different growth and differentiation stages of the cell, as well as according to its type and volume. A typical cell has a macromolecular concentration in the range 100-450 g/L, with 5-40% of its volume being occupied by macromolecules [1]. Therefore, the space available for the free diffusion of metabolites and other macromolecules is greatly reduced, leading to what is known as an excluded volume effect. The slow diffusion of macromolecules under crowded conditions has been explained using transient complex formation [2]. However, the role played by these two individual components, namely transient complex formation and excluded volume effects, in slowing the diffusion process is not well characterized. More importantly, sub-diffusive behaviour exhibited by macromolecules under crowded conditions and its causes need to be further investigated. In this study we used a molecular dynamics simulation approach to characterise the diffusion of chymotrypsin inhibitor 2 (CI2) in protein solutions of bovine serum albumin (BSA) and lysozyme at concentrations ranging from 50 to 300 g/L [3]. These simulations were found to be consistent with the changes in diffusion coefficient as a function of crowder concentration observed in NMR experiments. These simulations indicate that sub-diffusive behaviour observed in the sub-microsecond timescale can be explained in terms of a caging effect. The simulations were performed by selectively manipulating the nature of interactions between protein molecules, underlining the importance of excluded volume effects in explaining slow diffusion in crowded protein solutions.

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Perfringolysin O pore formation dynamics: from soluble monomer to membrane insertion

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Perfringolysin O (PFO) is a cholesterol dependent cytolysin (CDC) secreted by *Clostridium perfringens*, which forms pores in cholesterol containing membranes. CDCs are part of the larger Membrane attack complex-Perforin/CDC (MACPF/CDC) superfamily, containing pore formers responsible for controlling infectious disease and cancer in humans. MACPF/CDCs are secreted as soluble hydrophilic monomers which oligomerise on lipid bilayers, ultimately forming bilayer spanning ring or arc-shaped β-barrel pores. Perfringolysin O (PFO) was the first CDC to have its crystallographic structure resolved in its soluble monomeric form and has since become the prototypical CDC for investigating pore-forming mechanism [2].

Previous studies on PFO have revealed a general outline of the steps involved in MACPF/CDC pore formation; recognition of cholesterol and membrane binding, oligomerisation and ultimately membrane insertion to form large amphipathic pores. These steps have been elucidated using bulk assays and static imaging techniques such as electron microscopy or atomic force microscopy, however key mechanistic details remain uncharacterised due to the lack of time resolved data at a single pore level. Here we present a novel method using total internal reflection microscopy to track PFO pore formation dynamics. Fluorescently labelled PFO and dye encapsulating liposomes were employed in conjunction to measure the kinetics of PFO binding from solution, nucleation and oligomerisation on the surface of cholesterol containing liposomes. By visualising fluorescent dye release from our liposomes, we were able to determine the number of molecules necessary for an oligomer to insert and form a bilayer spanning pore.

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Reconstitution of recombinant human AQP1 into lipid vesicles enables single-channel recordings of AQP1 cation channel activity.

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Human aquaporin (AQP1) forms homotetrameric channels that facilitate water and small solute fluxes across cell membranes. Moreover, AQP1 displays a cyclic GMP (cGMP)-gated non-selective monovalent cation channel activity that may contribute towards regulating cell shape, volume, and motility. However, further work is still needed to define the physiological role of AQP1 dual water and ion conductivity. In this study, AQP1 ion conductivity was investigated using proteoliposomes which, unlike conventional cell-based systems such as oocytes, are free of background electrogenic channels. Histidine-tagged recombinant AQP1 protein was synthesised and purified from the methylotrophic yeast *Pichia pastoris*. Water channel function of AQP1-His_6 in liposomes was confirmed by stopped-flow spectroscopy. Ion channel activity of AQP1-His_6 was then assessed using electrophysiology of excised patches in the inside out configuration. In symmetrical potassium the AQP1-His_6 channels displayed coordinated gating, single channel conductances of around 100 pS, as well as multiple sub-conductance states. Channel activity was reduced when 600 µM $CdCl_2$ solution was added to the bath, which is consistent with previous studies showing inhibition of AQP1 by divalent cations [1]. This novel method opens new avenues for studying the structure-function relationships of aquaporin ion channels, and for discovering new inhibitors of AQP1 ion channels that could control cell morphology and inhibit cancer cell migration.

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Formation of a novel two-component pore complex by a homologous pair of pore-forming toxins from *Elizabethkingia anophelis*

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Cholesterol-dependent cytolysins (CDCs) are bacterial pore-forming toxins that are secreted as soluble monomers and oligomerise into large circular pre-pores on the surface of cholesterol-rich membranes. Various structural changes and transitions results in insertion of β-hairpins into the lipid bilayer, forming a large β-barrel pore that results in cell lysis [1]. We have identified a highly conserved structural motif of CDCs that plays a critical role in the prepore-to-pore transition. Furthermore, this motif is also highly conserved in a large, diverse family of uncharacterised proteins from over 220 species, which we have designated the name "CDC-like" (CDCL) proteins [2]. Many of these CDCLs exist as homologous pairs. One partner of the CDCL pair, termed CDCL long, consists of four domains: three similar to CDCs and a unique fourth domain. The other partner, CDCL short, possesses three domains, all similar to CDCs. We have identified a novel CDCL pair, referred to as ALY long (ALY^L) and ALY short (ALY^S), that originates from the species *Elizabethkingia anophelis,* a commensal bacterium of the Anopheles mosquito. X-ray crystallography revealed the structure of monomeric ALY^L consists of characteristic CDC domain 1 – 3 structure; however, domain 4 differs from that of CDCs significantly. In the presence of lipids, ALY^L and ALY^S show weak pore-forming activity and analysis by negative-staining electron microscopy reveals a large circular oligomeric complex reminiscent of CDC pore complexes. ALY^S also forms a non-lytic pore-like oligomer in the absence of ALY^L with further investigation suggesting ALY^S to be the membrane-inserting subunit of the heterocomplex. XL-MS data reveals a putative binding interface between ALY^S and ALY^L in addition to providing an initial insight into the structural changes between the monomeric and heterooligomeric states, which will be further supported by the high-resolution cryo-EM structure of the complex. In summary, we have shown using multiple structural techniques that the toxins share some structural resemblance to CDCs, but also form a two-component pore complex that is unique to the CDC family. This study establishes the beginning of an investigation into the large family of novel CDC-like proteins present in a wide range of bacterial species and are suspected to play key roles in microbial survival and human disease.

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Session 8: Viral Biophysics/ McAuley Hope

15:00-17:00

Mechanism and inhibition of the papain-like protease, PLpro, of SARS-CoV-2

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Coronaviruses, including SARS-CoV-2, encode multifunctional proteases that are essential for viral replication and evasion of host innate immune mechanisms. The papain-like protease PLpro cleaves the viral polyprotein and reverses inflammatory ubiquitin and anti-viral ubiquitin-like ISG15 protein modifications. Drugs that target SARS-CoV-2 PLpro (SARS2 PLpro) may hence be effective as treatments or prophylaxis for COVID-19 reducing viral load and reinstating innate immune responses.
In our study we characterise SARS2 PLpro in molecular and biochemical detail. SARS2 PLpro cleaves ISG15 modifications with high activity, as well as, Lys-48-linked polyubiquitin with lower activity. Structures of PLpro bound to ubiquitin and ISG15 reveal that the S1 ubiquitin-binding site is responsible for high ISG15 activity, while the S2 binding site provides Lys48 chain specificity and cleavage efficiency. We exploit two repurposing approaches to target SARS2 PLpro: A high throughput screen against SARS2 PLpro using a library of 3,727 unique approved drugs and clinical compounds identified no compounds that inhibited PLpro consistently or that could be validated in counter screens. More promisingly, non-covalent small molecules SARS PLpro inhibitors, prevent self-processing of nsp3 in cells and display high potency and excellent antiviral activity in a SARS-CoV-2 infection model.

Re-engineering of SARS-CoV-1 monoclonal antibodies for SARS-CoV-2 neutralization

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The SARS-CoV-2 coronavirus, responsible for the COVID-19 global pandemic, is threatening humankind with over 50 millions cases reported thus far. Until an effective vaccine is discovered and available, alternative therapies may limit the spread of the pandemic. Monoclonal antibodies represent promising candidates to reduce viral-related symptoms as well as viral loads, as demonstrated with Synagis, the first FDA approved monoclonal antibodies to treat RSV in children at risk. Some SARS (CoV-1) neutralising antibodies have been identified during the 2003 outbreak, the majority blocking the receptor binding domain of the spike protein to interact with the human Ace2 receptor. Considering the high sequence homology between the receptor binding domain of SARS and SARS-CoV-2, we aimed to re-engineer four existing antibodies (CR3022, CR3014, m396 and 80R) to neutralise CoV-2. We employed site-directed mutagenesis in the CDR regions and light chain shuffling as two complementary strategies for the re-engineering and generated phage display antibody libraries. After selection using CoV2 RBD, we identified a series of neutralising antibodies with IC_{50} ranging from 50 to 0.2 µg/ml. Importantly, we showed that site-directed mutagenesis can generate antibodies binding the same epitope as the parental it originated from. Using X-ray crystallography and cryo-EM, we demonstrated that light chain shuffled derived antibodies have switched epitopes with light chain dominated antigen binding. Overall, we have generated antibody phage libraries that allowed the identification of SARS-CoV-2 neutralising antibodies and that may be used for future coronavirus pandemics.

Towards understanding strain variances in coronavirus pathogenicity by simulating S Protein RBD energetics.

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SARS-CoV-2, the virus inducing the COVID-19 disease, has claimed over 1.1 million lives since emerging in December 2019, while disrupting many more. It is the seventh coronavirus to emerge in human populations. However, what makes one coronavirus more deadly than another is not well understood. Furthermore, no tools exist to reliably assess pathogenic potential in coronavirus strains threatening future emergence from animal populations. Here, we attempt to answer why one coronavirus is more pathogenic than another and describe a potential tool for future predictive uses. Coronaviruses invade host cells using a spike glycoprotein. To bind to the host receptor and initiate fusion with the host membrane, the spike protein's receptor binding domain (RBD) must first transition from a closed to open conformation to avail itself for binding [1,2,3]. We propose that the proportion of time spent in the open receptor-ready conformation, related to this conformation's stability, indicates the pathogenic potential of the coronavirus strain. To test this hypothesis, we compared the spike protein RBDs from three human ACE2 binding coronavirus strains with varying pathogenic potential: SARS-CoV-1, SARS-CoV-2, and hCoV-NL63, using molecular dynamics simulations. We employed both long equilibrium simulations and umbrella sampling to derive closed to open transition free energy profiles. While preliminary, our results suggest some correlation between the ability of the RBDs to transition to the open state and pathogenic potential. With additional testing, our technique may prove useful in assessing animal coronaviruses' pathogenic potential, highlighting them as a high or low-risk strain before they can emerge into human populations.

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