PI profile

## Shoumo Bhattacharya

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### GMS themes:

* Genomic analysis (bioinformatics and statistical genetics)
* From genes to clinic (target discovery, structural biology, medicinal chemistry)
* Application of genomics in the clinic (diagnostics and therapeutics)

### Research Overview

Protein interactions underlie most biological processes but are challenging to target using small molecule drugs. Many protein interactions are mediated by short-linear-interacting motifs (SLiMs) that range from 3-12 amino acid residues (1, 2). Small peptides designed from SLiMs can disrupt specific protein interactions and are broadly applicable to therapeutic development. A classic example is the ACE inhibitor drug series (e.g. captopril) that originated in the snake venom peptide teprotide. Our lab focusses on identifying SLiMs that mediate chemokine protein interactions. Chemokines are secreted signalling peptides that are produced by diseased or injured tissues - and underlie not only acute disease (e.g. COVID-19 lung inflammation) but also many chronic immuno-inflammatory diseases such as ulcerative colitis and rheumatoid arthritis. The 46 chemokines signal through 19 GPCRs to activate leucocyte (e.g. neutrophil, monocyte, T-cell) migration (3). The redundancy of the chemokine network with a single chemokine being able to target multiple receptors, and multiple receptor classes being expressed on leucocytes results in network robustness. This means that a single point of attack fails to effectively neutralize the chemokine network in disease. Natural chemokine-binding proteins (CKBPs) from ticks and viruses bind *multiple* chemokines, blocking chemokine-receptor binding, chemokine-dimerization, and chemokine-glycosaminoglycan binding, overcoming redundancy (4). CKBPs are effective in several disease models of inflammation, and as inflammation imaging agents. Work from our and other labs indicate that CKBP:chemokine, chemokine:chemokine, and chemokine:receptor interactions are mediated by short-linear-interacting-motifs (SLiMs) and that synthetic SLiM-peptides bind multiple chemokines and inhibit chemokine function (5, 6). These SLiM-peptide properties suggest their potential for development as *theranostic* (*thera*peutic and diag*nostic*) agents in immuno-inflammatory diseases.

Over 500 CKBP:chemokine, chemokine:chemokine, chemokine:glycosaminoglycan and chemokine:receptor interactions are known, precluding use of traditional structural biology approaches to identify the molecular mechanism of interaction. To systematically identify SLiMs that mediate these interactions we are using a phage display -nextGen sequencing (NGS) approach (2). Known CKBPs, chemokines and chemokine receptors have been deconstructed into overlapping 16-mer peptides that are expressed on phage. Binding of specific peptides to a target (e.g. a chemokine) is detected by affinity purification of phage bound to the target. Bound phage are sequenced using NGS and peptides enriched over the input (SLiMs) are identified using a series of custom-written R scripts. Synthetic SLiM-peptides are then used to validate the binding, determine binding affinity, and assay effect on chemokine function cell migration. These experiments provide an understanding of the molecular mechanisms by which CKBPs, chemokines and receptors display “one-to-many" binding interactions. The phage-display NGS approach can also be used to mutagenize and combine peptides to achieve optimal therapeutic efficacy. The phage-display NGS approach is broadly applicable to identifying the molecular basis of other protein interactions for instance in the cytokine network or the interaction of viral oncogenes with host proteins.

Project areas: protein interactions, inflammation, chemokine, phage-display, next-generation sequencing, theranostics

### Specific project proposals:

* ‘Identifying the molecular basis of chemokine:receptor binding using phage-display and application to peptide theranostic development’
* ‘Identifying the molecular basis of chemokine oligomerization using phage-display and application to peptide theranostic development’

Please contact directly for further information.

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Project proposal

Title: Identifying the molecular basis of chemokine:receptor binding using phage-display and application to peptide theranostic development’

Supervisors: Shoumo Bhattacharya, Graham Davies

Wet/dry lab mix (approx): 70% wet lab, 30% dry lab

### Description: The 24 chemokine receptors are GPCRs that either cause leucocyte migration or act as ‘sumps’ to neutralize chemokines (3). The molecular basis of these interactions will be decoded by screening a receptor phage-display library with matrix attached chemokines and analysed by NGS. SLiM-peptides identified will be studied for biochemical (e.g. binding to target by fluorescent polarisation, protein-interaction disruption using bead-capture assays) and biological activity (e.g. receptor dimerization using proximity-ligation assay, cell migration assays).

### Training Opportunities:

Molecular biology, cloning, protein interactions, phage-display, analysis of NGS datasets, R scripting, network analysis, cell biology, chemotaxis, flow cytometry.

### Background reading / references:

1. Ivarsson, Y., and Jemth, P. (**2019**). Affinity and specificity of motif-based protein-protein interactions. *Curr Opin Struct Biol* 54**,**26-33, 10.1016/j.sbi.2018.09.009

2. Mclaughlin, M.E., and Sidhu, S.S. (**2013**). Engineering and analysis of peptide-recognition domain specificities by phage display and deep sequencing. *Methods Enzymol* 523**,** 327-349, 10.1016/B978-0-12-394292-0.00015-1

3. Kufareva, I., Gustavsson, M., Zheng, Y., et al. (**2017**). What Do Structures Tell Us About Chemokine Receptor Function and Antagonism? *Annu Rev Biophys* 46**,** 175-198, 10.1146/annurev-biophys-051013-022942

4. Bhusal, R.P., Eaton, J.R.O., Chowdhury, S.T., et al. (**2020**). Evasins: Tick Salivary Proteins that Inhibit Mammalian Chemokines. *Trends Biochem Sci* 45**,** 108-122, 10.1016/j.tibs.2019.10.003

5. Darlot, B., Eaton, J.R.O., Geis-Asteggiante, L., et al. (**2020**). Engineered anti-inflammatory peptides inspired by mapping an evasin-chemokine interaction. *J Biol Chem* 295**,** 10926-10939, 10.1074/jbc.RA120.014103

6. Von Hundelshausen, P., Agten, S.M., Eckardt, V., et al. (**2017**). Chemokine interactome mapping enables tailored intervention in acute and chronic inflammation. *Sci Transl Med* 9, 10.1126/scitranslmed.aah6650

Project proposal

Title: Identifying the molecular basis of chemokine oligomerization using phage-display and application to peptide theranostic development’

Supervisors: Shoumo Bhattacharya, Graham Davies

Wet/dry lab mix (approx): 70% wet lab, 30% dry lab

Description: Chemokine oligomerization is critical for function (6). The molecular basis of these interactions will be decoded by screening a chemokine phage-display library with matrix attached chemokines and analysed by NGS. SLiM-peptides identified will be studied for biochemical (e.g. binding to target by fluorescent polarisation, protein-interaction disruption using bead-capture assays) and biological activity (e.g. receptor dimerization using proximity-ligation assay, cell migration assays).

### Training Opportunities:

Molecular biology, cloning, protein interactions, phage-display, analysis of NGS datasets, R scripting, network analysis, cell biology, chemotaxis, flow cytometry.

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1. Ivarsson, Y., and Jemth, P. (**2019**). Affinity and specificity of motif-based protein-protein interactions. *Curr Opin Struct Biol* 54**,**26-33, 10.1016/j.sbi.2018.09.009

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