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In vivo systems biology approaches to chronic immune/inflammatory pathophysiology

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Systems biology offers an emphasis on integrative computational analysis of complex multi-component processes to enhance capability for predictive insights concerning operation of those processes. The immune system represents a prominent arena in which such processes are manifested for vital roles in physiology and pathology, encompassing dozens of cell types and hundreds of reciprocal interactions. Chronic, debilitating pathologies involving immune system dysregulation have become recognized as increasing in incidence over recent decades. While clinical consequences of immune dysregulation in such pathologies are well characterized, treatment options remain limited and focus on ameliorating symptoms. Because it is difficult to recapitulate more than a severely limited facet of the immune system *in vitro*, application of systems biology approaches to autoimmune and inflammatory *pathophysiology in vivo* has opened a new door toward discerning disease sub-groups and developing associated stratification strategies for patient treatment. In particular, early instances of these approaches have demonstrated advances in uncovering previously under-appreciated dysregulation of signaling networks between immune system and tissue cells, raising promise for improving upon current therapeutic approaches.

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Introduction

Rather than being regulated by a set of exact parameters, each individual's immune system evolves under a set of loose biological guidelines, resulting in vast person-to-person variability [1,2•]. This biological noise makes it difficult to separate normal variation from genuine disease drivers using conventional analyses [3]. Systems biology concepts and methods provide multi-variate approaches to holistically analyze the larger interactive network of biological pathways and identify important players in inflammatory disease onset and progression.

The limitation of *ex vivo* and reductionist, univariate approaches in studies of inflammatory disease is the focus on previously defined immune cell populations and signaling interactions. Foundational work in immunology defines some subsets of immune cells as more or less important based solely on their surface markers through flow cytometry analysis. The cells within these subsets are often treated as homogenous and fixed in identity, which is untrue *in vivo* [4,5]. Immune cells are plastic [6,7•], and thus their interactions generate dynamic signaling networks, among themselves and with cells of tissues within which they reside or into which they migrate [8•]. Thus, a systems approach that considers the full spectrum of *in vivo* cell–cell signaling interactions is the only one that can hope to identify drug targets that will more probably make it through clinical trials.

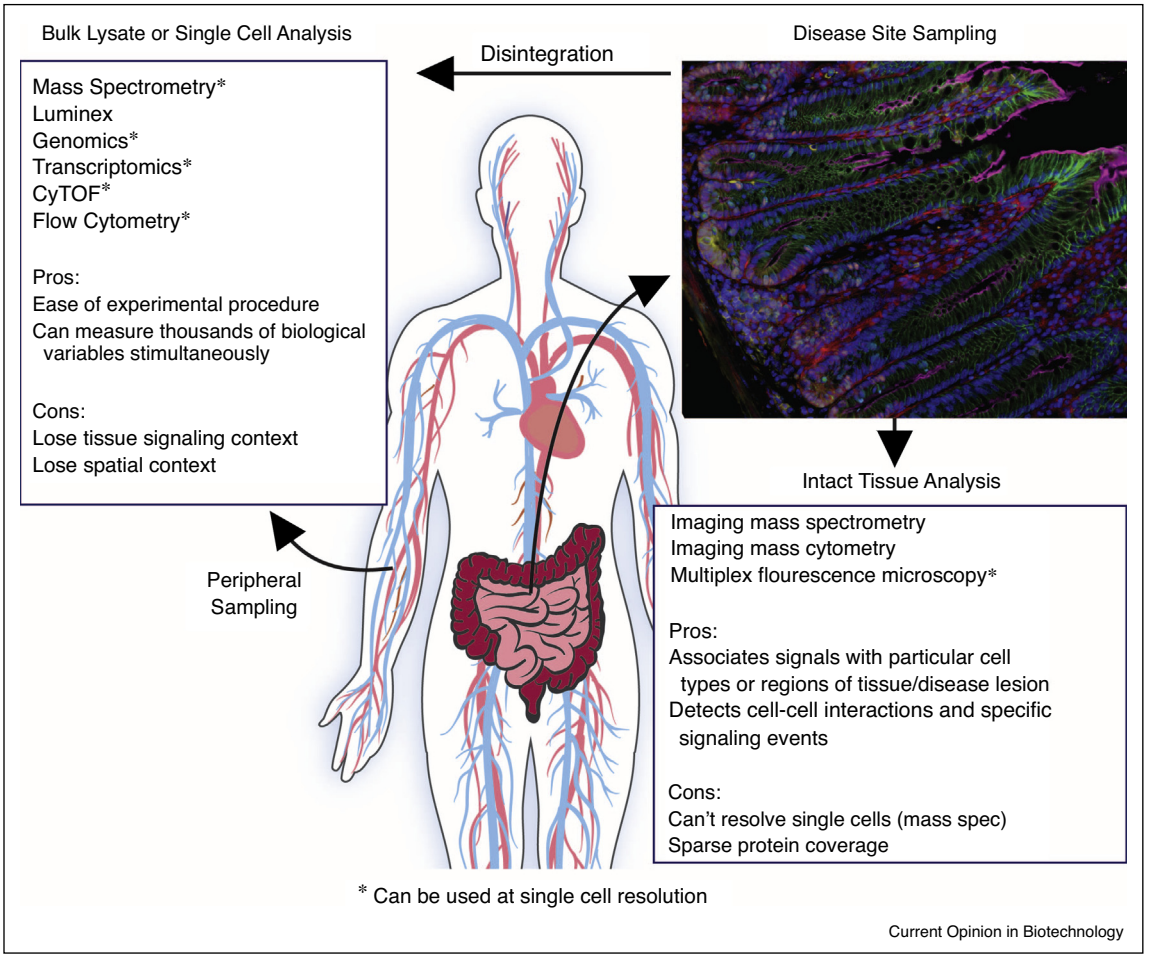
In vivo systems approaches identify biomarkers to better diagnose human disease

A large effort has been put forth to identify biomarkers for diagnosis and stratification of patients for treatment. In the early days of genomic sequencing, GWAS (genome-wide association studies) promised to provide the information necessary to find populations at risk for a given disease, predict drug responsiveness, and identify disease subgroups. Although some genetic variations have been associated with inflammatory diseases such as Rheumatoid arthritis (RA), Inflammatory Bowel disease (IBD), Lupus Systemic Erythematous (SLE) and others [9,10], these studies have not been as clinically transformative as hoped, with many patients displaying physiological pathologies but not possessing the associated gene variants. Further, GWAS have succeeded mostly in identifying correlated genes rather than targetable drivers [11].

Searching for peripheral biomarkers

Advances in transcriptomic and proteomic technologies (Figure 1) have allowed systems biologists to pursue biomarker identification using *in vivo* systems biology approaches [12,13••,14••,15–18,19•,20•,21••]. Two categories of patient-derived *in vivo* samples are available for such research: fluids and tissues. Peripheral blood analysis is optimal due to its ease of integration into existing clinical procedures. Use of peripheral blood transcriptomics combined with a mixed modeling approach has led to models that stratify patients into sub-groups in SLE

Figure 1



Approaches to generating biological data for subsequent *in vivo* systems biology analysis.

and IBD. Such models identified several unrecognized subtypes of SLE and retrospectively predicted responses to existing treatments based on network parameters [14••,19•]. The importance of measuring temporal fluctuations of *in vivo* signaling networks in autoimmune disease has also been highlighted and used to expand the IFN signature canonically thought to associate with SLE [19•].

The flexibility of systems analyses allows for combination of biological data with clinical parameters. This ability to combine heterogeneous data types allows for studies to identify molecular targets associated with particular clinical outcomes and to stratify patients based on either or both of these criteria [12,17]. Measurement of biological features in patient-derived fluids has identified subgroups within some inflammatory diseases, but has been largely unsuccessful in peripheral samples from IBD [22]. The latter highlights a potential limitation of sampling from a peripheral site: increased 'distance' between peripheral blood and the primary disease site leads to dispersion of disease-specific biological material to the point where detection of the marker may fall to background levels.

Identification of biomarker states in the primary disease site

The dispersion problem can be solved through direct analysis of affected tissues (Figure 1). This approach is technically more challenging, but directly samples the signaling networks driving disease pathophysiology. Studies of intact tissue allow for analysis of interactions between immune cells and affected solid tissue cell types, whose destruction or malfunction results in patient symptoms. Signaling within tissues can be interrogated through either homogenization followed by –omics techniques, or disintegration into single cells followed by CyTOF [23,24], flow cytometry [25], or single cell –omics [26,27,28•]. A drawback of tissue dissociation is that it alters signaling of normally adherent cells within minutes [29]. Recent protocols have been developed to address

these issues, either through changes in the dissociation protocols [24] or through use of stochastic profiling, which is compatible with tissue processing techniques that minimize handling artifacts in the resulting pool of single cells [30]. Alternatively, imagining techniques that utilize tissue sections and retain spatial resolution of the sample can be harnessed to study cell–cell interactions [31–33]. These techniques have not yet been applied to studies of autoimmunity and inflammation, but their success in studies of pathologies in the same organ systems [34,35] make us confident in their future usefulness (Figure 2).

Application of *in vivo* systems biology to biomarker identification in inflammatory diseases has revealed their underlying molecular heterogeneity [12,14••,19•,36–38]. These results may explain the failures of multiple clinical trials and the low response rate to existing targeted therapies. Further, they provide rationale for studying each disease driven by immune dysregulation as a set of molecularly different diseases with a similar pathophysiological outcome rather than as a single disease that can be treated with a single therapeutic approach.

Application focus: identifying drug targets

Current approaches to identify putative drug targets have not resulted in significant improvements to treatment of autoimmune and inflammatory diseases. Some, like SLE, have not seen major changes in treatment options in the past 50 years. Systems-based interrogation of *in vivo* signaling networks is beginning to predict useful drug targets by identifying previously unappreciated interactions in more physiologically relevant signaling networks.

Mapping signaling networks through perturbations *in vivo*

Historically, *in vivo* systems biology originated in signaling studies performed in murine models of inflammatory signaling. Early work used a multi-variate modeling approach to deconvolute the effects of TNF-a, a ligand known to stimulate both survival and apoptosis in a receptor-specific fashion [39]. The results of these early studies were astounding: not only was there a dose-dependent temporal control over cell response to TNF-a, but different regions of the intestine responded differently to the ligand [40,41]. Follow up studies utilized a similar approach to identify a set of kinase inhibitors to modulate these responses *in vivo* [42••]. These studies demonstrate the dynamic and adaptive nature of signaling networks that is lost outside the *in vivo* context.

Signaling studies with human tissue

Mouse signaling networks do not generally translate directly to human ones [3,43–45]. Thus, some studies have focused on perturbation experiments in patient-derived tissues *ex vivo*. Multivariate analysis of signaling in patient-derived tissues in RA has identified defects in B-cell signaling through defective cytokine production [46•] and determined how cytokine mis-expression contributes to disease progression through activation of synovial fibroblasts [13••]. The caveat with these studies is that primary cells grown *in vitro* for even a short time differ from their *in vivo* counterparts, as determined through multivariate systems analysis of monocyte differentiation derived from human subjects [29]. Another methodology for gleaning *in vivo* information from publically available patient and murine data represents how groups without access to mouse or patient samples can use systems approaches to generate or support hypotheses [47].

Mapping signaling networks in humans

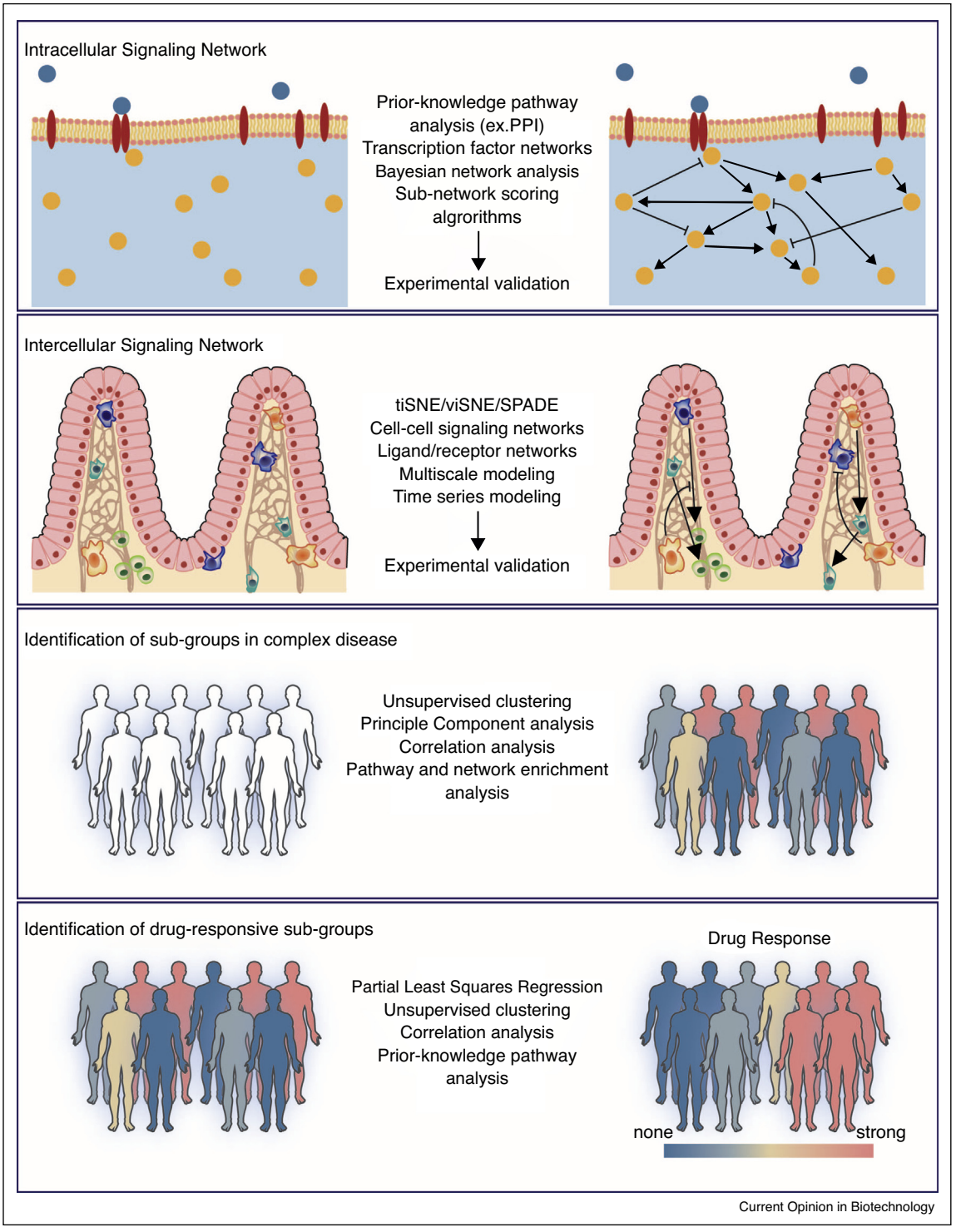
Two recent studies provide a blueprint and a rationale to probe human immune system signaling *in vivo*. In one, peripheral blood from Celiac patients exposed to a gluten challenge was sampled over time and analyzed through a combination of flow cytometry, CyTOF and single cell sequencing. The result identified coordinated responses between different T-cell types and suggests their involvement in the destruction of intestinal epithelial cells. This study also sheds light on how initial misdirection of CD4+ T-cells may cause effector T-cell mediated tissue destruction [48••]. While treating human subjects with disease-activating stimuli is ethically challenging, applying this approach to patients prior to and after targeted drug treatment would provide invaluable insights into disease-driving signaling networks and may identify network components that associate with responsiveness or side effects, thereby bettering patient quality of life [48••,49].

Computational approaches: 'So you want to use systems analyses . . . '

Many approaches exist to explore the structure of interconnected, multi-dimensional data such as that utilized by the studies discussed above. Three basic areas involved in the modeling of *in vivo* signaling networks include machine learning, graph theory and non-linear dynamics and chaos. We cannot hope to adequately cover these, but excellent reviews and perspectives can be found here [28•,50,51]. Instead, we provide an example set of steps to begin exploring multivariate data [52].

Analysis often begins with hierarchical clustering as an exploratory method to provide a picture of the landscape of similarity and grouping of samples based on given measurements [53,54]. Machine learning multivariate projection approaches such as principle component analysis (PCA) and partial least squares (PLS) (PLS can be implemented as regression, for continuous responses, or discriminant analysis, for discrete states) can simultaneously provide insight into which measurements (protein, cytokine, cell type, and so on) or patterns of

Figure 2



Common systems biology approaches for particular problems in human health.

covarying measurements correlate with a given phenotype [55–57]. The resulting models can be built upon using prior knowledge pathway analysis, which draws from curated pathway databases like KEGG [58] and GO to aid in extraction of biologically relevant interactions. Two commonly used tools for this extraction are Gene Set Enrichment Analysis (GSEA) and ALIGATOR [59,60]. Causal relationships can be determined using

Bayesian networks, which combine prior knowledge with data from the current experiment [61–63].

Together, these techniques can identify features of interest in a given network of biological interactions between immune cells and tissue, for example those that lead to an IBD lesion. Results from these studies must be properly cross-validated and require experimental follow-up, often implemented as an iterative process using computation to inform experiment and vice versa, leading to an understanding of not only how an initial disease-associated signaling networks differ from healthy ones, but how perturbation of a given target (cytokine, receptor, cell type) can alter the disease network to more closely resemble the healthy one. If the above looks daunting, note that one does not need all of these methods and that fairly simple approaches can be used to begin drawing interesting and testable observations from multivariate data.

Final thoughts: looking toward the future

Newly developed technologies are generating new types of data for systems analysis. Advances in mass spectrometry, imaging and sample processing promise to transform our ability to study cell–cell signaling *in vivo*. Mass spectrometry has been instrumental in providing data for systems analysis. Recent advances provide means to reduce required sample size for signaling studies [26], to detect protein interaction partners and to identify putative new phosphorylation sites [64]. Analysis of solid tissue has been hindered by its structural complexities. New protocols for disintegration of solid tissues and subsequent analysis by CyTOF will provide single cell resolution to solid tissue signaling networks [23,24], making the identification of disease-specific cell populations and the signaling events they participate in possible.

Advances in spatial visualization of proteins in a tissue through spatially oriented mass spectrometry provide an alternate solution to the solid tissue problem, and allow for direct observation of immune cell–target cell interactions [49,65–67]. Use of cyclic immunofluorescent approaches preserves the tissue sample for further analysis [34] thus theoretically allowing for multiple analyses from the same sample. A current disadvantage of the intact tissue approaches is the low number of measurements that can be generated (dozens) when compared to –omics approaches (thousands). However, these antibody-based can be coupled with antibodies specific not only to modifications such as phosphorylation, but also ones that detect protein complexes and conformational changes to add an extra layer of information [68]. Signaling by cells and within cells is complex and variable by tissue and cell type. The more layers of information gleaned about the network, the better it can be defined and the more likely we are to identify the features driving specific disease phonotypes.

Immunology-specific technical advances have begun to lower barriers to *in vivo* inflammation research. The substitution of traditional and limited gating strategies with viSNE and SPADE algorithms has allowed for identification of small cell populations and revealed elements of inflammatory network plasticity that are lost in methods employing univariate, non-single cell analysis [18,69–71]. The discovery that immune cells secrete and signal through exosomes also provides a new method of sampling peripheral blood by allowing for the concentration of biologically active particles, rich in protein and RNA components [72,73]. Plasma-derived exosomes are already being studied as putative biomarkers in several inflammatory pathologies. Whether human or murine, *in vivo* systems studies are expensive. Advances in microphysiological 'organ on a chip' platforms that include immune cells as well as several interacting components show promise in reducing costs and making temporal analysis possible [74].

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Brodin P, Davis MM: **Human immune system variation**. *Nat Rev Immunol* 2016, **17**:21-29.

2. • Brodin P, Jojic V, Gao T, Bhattacharya S, Angel CJL, Furman D, Shen-Orr S, Dekker CL, Swan GE, Butte AJ *et al.*: **Variation in the human immune system is largely driven by non-heritable influences**. *Cell* 2015, **160**:37-47.

The authors discuss how person-to-person immune system variation arises and provide an excellent review of the progress in systems immunology with a focus on responses to environmental challenge.

3. von Herrath MG, Nepom GT: **Lost in translation: figure 1**. *J Exp Med* 2005, **202**:1159-1162.

4. Sica A, Mantovani A: **Macrophage plasticity and polarization: in vivo veritas**. *J Clin Invest* 2012, **122**:787-795.

5. DuPage M, Bluestone JA: **Harnessing the plasticity of CD4+ T cells to treat immune-mediated disease**. *Nat Rev Immunol* 2016, **16**:149-163.

6. McDonald PW, Read KA, Baker CE, Anderson AE, Powell MD, Ballesteros-Tato A, Oestreich KJ: **IL-7 signalling represses Bcl-6 and the TFH gene program**. *Nat Commun* 2016, **7**:10285.

7. • Laurent P, Jolivel V, Manicki P, Chiu L, Contin-Bordes C, Truchetet M-E, Pradeu T: **Immune-mediated repair: a matter of plasticity**. *Front Immunol* 2017, **8**:454.

The authors provide rationale for holistic approaches to immunology and review evidence for the immune cell plasticity concept.

8. • Subramanian N, Torabi-Parizi P, Gottschalk RA, Germain RN, Dutta B: **Network representations of immune system complexity**. *Wiley Interdiscip Rev Syst Biol Med* 2015, **7**:13-38.

The authors detail different types of network modeling and how these can contribute to our understanding of the immune system. And excellent overview of different types of networks referred to in the studies discussed here.

9. Fodil N, Langlais D, Gros P: **Primary immunodeficiencies and inflammatory disease: a growing genetic intersection**. *Trends Immunol* 2016, **37**:126-140.

10. Rivas MA, Beaudoin M, Gardet A, Stevens C, Sharma Y, Zhang CK, Boucher G, Ripke S, Ellinghaus D, Burtt N *et al.*: **Deep resequencing of GWAS loci identifies independent rare variants associated with inflammatory bowel disease**. *Nat Genet* 2011, **43**:1066-1073.

11. Ku CS, Loy EY, Pawitan Y, Chia KS: **The pursuit of genome-wide association studies: where are we now?** *J Hum Genet* 2010, **55**:195-206.

12. Jørgensen H, Hill AS, Beste MT, Kumar MP, Chiswick E, Fedorcsak P, Isaacson KB, Lauffenburger DA, Griffith LG, Qvigstad E: **Peritoneal fluid cytokines related to endometriosis in patients evaluated for infertility**. *Fertil Steril* 2017, **107** 1191–1199.e2.

13. •• Jones DS, Jenney AP, Swantek JL, Burke JM, Lauffenburger DA, Sorger PK: **Profiling drugs for rheumatoid arthritis that inhibit synovial fibroblast activation**. *Nat Chem Biol* 2016, **13**:38-45.
The authors analysed cytokine production of primary human sinovial fibroblasts upon activation with RA-associated cytokines which allowed them to build a model of cytokines important for SF activation and profile drugs that return SF output signals closer to their healthy state. This study highlights the utility of network analysis in both mechanistic and drug discovery studies.

14. •• Banchereau R, Hong S, Cantarel B, Baldwin N, Baisch J, Edens M, Cepika A-M, Acs P, Turner J, Anguiano E *et al.*: **Personalized immunomonitoring uncovers molecular networks that stratify lupus patients**. *Cell* 2016, **165**:551-565.
The authors longitudinaly profiles blood of SLE patients and utilized a mixed modeling approach to identify biomarkers for a variety of clinical outcomes. This study provides a prime example of how systems biology can be used to better uderstand factors underlying complex diseases and how this information can be invaluable to designing clinical studies for drugs that may only strongly benefit a portion of the total SLE population.

15. Abboud A, Mi Q, Puccio A, Okonkwo D, Buliga M, Constantine G, Vodovotz Y: **Inflammation following traumatic brain injury in humans: insights from data-driven and mechanistic models into survival and death**. *Front Pharmacol* 2016, **7**:342.

16. Cummings RJ, Barbet G, Bongers G, Hartmann BM, Gettler K, Muniz L, Furtado GC, Cho J, Lira SA, Blander JM: **Different tissue phagocytes sample apoptotic cells to direct distinct homeostasis programs**. *Nature* 2016, **539**:565-569.

17. Shen-Orr SS, Furman D, Kidd BA, Hadad F, Lovelace P, Huang Y-W, Rosenberg-Hasson Y, Mackey S, Grisar FAG, Pickman Y *et al.*: **Defective signaling in the JAK-STAT pathway tracks with chronic inflammation and cardiovascular risk in aging humans**. *Cell Syst* 2016, **3** 374–384.e4.

18. Leelatian N, Diggins KE, Irish JM: **Characterizing phenotypes and signaling networks of single human cells by mass cytometry**. *Methods in Molecular Biology*. Springer; 2015:99-113.

19. • Chiche L, Jourde-Chiche N, Whalen E, Presnell S, Gersuk V, Dang K, Anguiano E, Quinn C, Burtey S, Berland Y *et al.*: **Modular transcriptional repertoire analyses of adults with systemic lupus erythematosus reveal distinct type I and type II interferon signatures**. *Arthritis Rheumatol* 2014, **66**:1583-1595.
The authors samlped SLE patients prospectively and used modular repertoire analysis to identify a temporally complex and varied signature of IFN in SLE. This study demonstrates the dynamic nature of *in vivo* immune disease-associated networks.

20. • Fleischer S, Ries S, Shen P, Lheritier A, Cazals F, Burmester GR, Dörner T, Fillatreau S: **Anti-interleukin-6 signalling therapy rebalances the disrupted cytokine production of B cells from patients with active rheumatoid arthritis**. *Eur J Immunol* 2017 http://dx.doi.org/10.1002/eji.201747191.
The authors detected differences in signaling networks between healthy and RA-derived B cells and utilized drugs to return the RA B cell network to a more healthy-like state. This study identifies B cell abnormalities in RA patients and provides putative biomarkers to predict response to certain RA theraputics.

21. •• Beste MT, Pfäffle-Doyle N, Prentice EA, Morris SN, Lauffenburger DA, Isaacson KB, Griffith LG: **Molecular network analysis of endometriosis reveals a role for c-Jun-regulated macrophage activation**. *Sci Transl Med* 2014, **6** 222ra16..
The authors measured cytokine profiles of peritoneal fluid derived from endometriosis patients and analyzed the resulting data using multivariate approaches. This is the first study to identify an endometriosis cytokine signature *in vivo*.

22. Bjerrum JT, Nyberg C, Olsen J, Nielsen OH: **Assessment of the validity of a multigene analysis in the diagnostics of inflammatory bowel disease**. *J Intern Med* 2014, **275**:484-493.

23. Leelatian N, Doxie DB, Greenplate AR, Mobley BC, Lehman JM, Sinnaeve J, Kauffmann RM, Werkhaven JA, Mistry AM, Weaver KD *et al.*: **Single cell analysis of human tissues and solid tumors with mass cytometry**. *Cytom Part B Clin Cytom* 2017, **92**:68-78.

24. Simmons AJ, Banerjee A, McKinley ET, Scurrah CR, Herring CA, Gewin LS, Masuzaki R, Karp SJ, Franklin JL, Gerdes MJ *et al.*: **Cytometry-based single-cell analysis of intact epithelial signaling reveals MAPK activation divergent from TNF-α-induced apoptosis in vivo**. *Mol Syst Biol* 2015, **11**:835.

25. Tang MS, Bowcutt R, Leung JM, Wolff MJ, Gundra UM, Hudesman D, Malter LB, Poles MA, Chen LA, Pei Z *et al.*: **Integrated analysis of biopsies from inflammatory bowel disease patients identifies SAA1 as a link between mucosal microbes with TH17 and TH22 cells**. *Inflamm Bowel Dis* 2017, **23**:1544-1554.

26. Humphrey SJ, Babak Azimifar S, Mann M: **High-throughput phosphoproteomics reveals in vivo insulin signaling dynamics**. *Nan Biotechnol* 2015 http://dx.doi.org/10.1038/nbt.3327.

27. Mohideen F, Paulo JA, Ordureau A, Gygi SP, Harper JW: **Quantitative phospho-proteomic analysis of TNFα/NFκB signaling reveals a role for RIPK1 phosphorylation in suppressing necrotic cell death**. *Mol Cell Proteom* 2017, **16**:1200-1216.

28. • Janes KA, Chandran PL, Ford RM, Lazzara MJ, Papin JA, Peirce SM, Saucerman JJ, Lauffenburger DA: **An engineering design approach to systems biology**. *Integr Biol Integr Biol* 2017, **574**:574-583.
This article offers a high-level discussion of an engineering approach to systems biology.

29. Roussel M, Ferrell PB, Greenplate AR, Lhomme F, Le Gallou S, Diggins KE, Johnson DB, Irish JM: **Mass cytometry deep phenotyping of human mononuclear phagocytes and myeloid-derived suppressor cells from human blood and bone marrow**. *J Leukoc Biol* 2017, **102**:437-447.

30. Wang L, Janes KA: **Stochastic profiling of transcriptional regulatory heterogeneities in tissues, tumors and cultured cells**. *Nat Protoc* 2013, **8**:282-301.

31. Lin J-R, Fallahi-Sichani M, Sorger PK: **Highly multiplexed imaging of single cells using a high-throughput cyclic immunofluorescence method**. *Nat Commun* 2015, **6**:8390.

32. Lin J-R, Fallahi-Sichani M, Chen J-Y, Sorger PK: **Cyclic immunofluorescence (CycIF), a highly multiplexed method for single-cell imaging**. *Current Protocols in Chemical Biology*. John Wiley & Sons, Inc.; 2016:251-264.

33. Nowicki TS, Akiyama R, Huang RR, Shintaku IP, Wang X, Tumeh PC, Singh A, Chmielowski B, Denny C, Federman N *et al.*: **Infiltration of CD8 T cells and expression of PD-1 and PD-L1 in synovial sarcoma**. *Cancer Immunol Res* 2017, **5**:118-126.

34. McKinley ET, Sui Y, Al-Kofahi Y, Millis BA, Tyska MJ, Roland JT, Santamaria-Pang A, Ohland CL, Jobin C, Franklin JL *et al.*: **Optimized multiplex immunofluorescence single-cell analysis reveals tuft cell heterogeneity**. *JCI Insight* 2017:2.

35. Simmons AJ, Scurrah CR, McKinley ET, Herring CA, Irish JM, Washington MK, Coffey RJ, Lau KS: **Impaired coordination between signaling pathways is revealed in human colorectal cancer using single-cell mass cytometry of archival tissue blocks**. *Sci Signal* 2016, **9** rs11-rs11.

36. Pawłowska J, Smoleńska Ż, Witkowski J, Bryl EM: **Different pattern of T-cell subpopulations in peripheral blood of patients with rheumatoid arthritis at various stages of disease development**. *Pol Arch Med Wewn* 2014, **124**:43-50.

37. Ince-Askan H, Hazes JMW, Dolhain RJEM: **Identifying clinical factors associated with low disease activity and remission of rheumatoid arthritis during pregnancy**. *Arthritis Care Res (Hoboken)* 2017, **69**:1297-1303.

38. O'Gorman WE, Hsieh EWY, Savig ES, Gherardini PF, Hernandez JD, Hansmann L, Balboni IM, Utz PJ, Bendall SC, Fantl WJ *et al.*: **Single-cell systems-level analysis of human Toll-like receptor activation defines a chemokine signature in patients with systemic lupus erythematosus**. *J Allergy Clin Immunol* 2015, **136**:1326-1336.

39. Gupta S: **A decision between life and death during TNF-alpha-induced signaling**. *J Clin Immunol* 2002, **22**:185-194.

40. Lau KS, Cortez-Retamozo V, Philips SR, Pittet MJ, Lauffenburger DA, Haigis KM: **Multi-scale in vivo systems analysis reveals the influence of immune cells on TNF-α-induced apoptosis in the intestinal epithelium**. *PLoS Biol* 2012, **10**:e1001393.

41. Lau KS, Juchheim AM, Cavaliere KR, Philips SR, Lauffenburger DA, Haigis KM: **In vivo systems analysis identifies spatial and temporal aspects of the modulation of TNF-α-induced apoptosis and proliferation by MAPKs**. *Sci Signal* 2011, **4** ra16-ra16.

42. •• Gierut JJ, Wood LB, Lau KS, Lin Y-J, Genetti C, Samatar AA, Lauffenburger DA, Haigis KM: **Network-level effects of kinase inhibitors modulate TNF-α-induced apoptosis in the intestinal epithelium**. *Sci Signal* 2015, **8** ra129-ra129.

The authors performed *in vivo* signaling studies on the intestinal signaling network in mice treated acutely with TNF- α in combination with kinase inhibitors and showed that drugs with similar targets differentially affect the *in vivo* signalling network. This study highlights that the state or context of a signaling network is as important as the pharmacological target in determining how a particular tissue will respond to a drug.

43. Davis MM: **A prescription for human immunology**. *Immunity* 2008, **29**:835-838.

44. Perlman RL: **Mouse models of human disease: an evolutionary perspective**. *Evol Med Publ Heal* 2016, **2016**:170-176.

45. Tjon ASW, van Gent R, Geijtenbeek TB, Kwekkeboom J: **Differences in anti-inflammatory actions of intravenous immunoglobulin between mice and men: more than meets the eye**. *Front Immunol* 2015, **6**:197.

46. • Rao DA, Gurish MF, Marshall JL, Slowikowski K, Fonseka CY, Liu Y, Donlin LT, Henderson LA, Wei K, Mizoguchi F *et al.*: **Pathologically expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis**. *Nature* 2017, **542**:110-114.

The authors analyze CD4+ T cells derived from inflammed joint tissue using a variety of systems techniques and identify a subset of peripheral helper T cells that may promote B cell activation and disease progression. This study utilizes unbiased systems biology to identify a previously overlooked population of T cells that may actually be a critical component of the immune network.

47. Kidd BA, Wroblewska A, Boland MR, Agudo J, Merad M, Tatonetti NP, Brown BD, Dudley JT: **Mapping the effects of drugs on the immune system**. *Nat Biotechnol* 2015, **34**:47-54.

48. •• Han A, Newell EW, Glanville J, Fernandez-Becker N, Khosla C, Chien Y-H, Davis MM: **Dietary gluten triggers concomitant activation of CD4+ and CD8+ αβ T cells and γδ T cells in celiac disease**. *Proc Natl Acad Sci U S A* 2013, **110**:13073-13078.

The authors treat patients with celiac disease with gluten and analyze subsequent changes in the immune network, showing that antigen induces CD8(+) αβ and γδ T cells in the peripheral blood. This study utilizes an *in vivo* systems approach in human subjects to identify key aspects of inflammatory disease pathology.

49. Hinkelbein J, Jansen S, Iovino I, Kruse S, Meyer M, Cirillo F, Drinhaus H, Hohn A, Klein C, Robertis E *et al.*: **Thirty minutes of hypobaric hypoxia provokes alterations of immune response, haemostasis, and metabolism proteins in human serum**. *Int J Mol Sci* 2017, **18**:1882.

50. Chuang H-Y, Hofree M, Ideker T: **A decade of systems biology**. *Annu Rev Cell Dev Biol* 2010, **26**:721-744.

51. Mitra K, Carvunis A-R, Ramesh SK, Ideker T: **Integrative approaches for finding modular structure in biological networks**. *Nat Rev Genet* 2013, **14**:719-732.

52. Wood LB, Winslow AR, Strasser SD: **Systems biology of neurodegenerative diseases**. *Integr Biol (Camb)* 2015, **7**:758-775.

53. Janes KA, Yaffe MB: **Data-driven modelling of signal-transduction networks**. *Nat Rev Mol Cell Biol* 2006, **7**:820-828.

54. D'haeseleer P: **How does gene expression clustering work?** *Nat Biotechnol* 2005, **23**:1499-1501.

55. Abdi H, Williams LJ: **Principal component analysis**. *Wiley Interdiscip Rev Comput Stat* 2010, **2**:433-459.

56. Eriksson L, Byrne T, Johansson E, Trygg J: **Multi- and megavariate data analysis basic principles and applications**. [date unknown].

57. Janes KA, Kelly JR, Gaudet S, Albeck JG, Sorger PK, Lauffenburger DA: **Cue-signal-response analysis of TNF-induced apoptosis by partial least squares regression of dynamic multivariate data**. *J Comput Biol* 2004, **11**:544-561.

58. Kanehisa M, Goto S, Furumichi M, Tanabe M, Hirakawa M: **KEGG for representation and analysis of molecular networks involving diseases and drugs**. *Nucleic Acids Res* 2010, **38**:D355-D360.

59. Holmans P, Green EK, Pahwa JS, Ferreira MAR, Purcell SM, Sklar P, Owen MJ, O'Donovan MC, Craddock N, Craddock N: **Gene ontology analysis of GWA study data sets provides insights into the biology of bipolar disorder**. *Am J Hum Genet* 2009, **85**:13-24.

60. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES *et al.*: **Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles**. *Proc Natl Acad Sci* 2005, **102**:15545-15550.

61. Ji R, Mitra R, Muller P, Qiu P: **Bayesian hierarchical models for protein networks in single-cell mass cytometry**. *Cancer Inform* 2014, **13**:79.

62. Larjo A, Shmulevich I, Lähdesmäki H: **Structure learning for bayesian networks as models of biological networks**. *Methods in Molecular Biology*. Springer; 2013:35-45.

63. Friedman N, Linial M, Nachman I, Pe'er D: **Using Bayesian networks to analyze expression data**. *J Comput Biol* 2000, **7**:601-620.

64. Supper V, Hartl I, Boulègue C, Ohradanova-Repic A, Stockinger H: **dynamic interaction- and phospho-proteomics reveal LCK as a major signaling hub of CD147 in T cells**. *J Immunol* 2017, **198**:2468-2478.

65. Heap RE, Hope AG, Pearson L-A, Reyskens KMSE, McElroy SP, Hastie CJ, Porter DW, Arthur JSC, Gray DW, Trost M: **Identifying inhibitors of inflammation: a novel high-throughput MALDI-TOF screening assay for salt-inducible kinases (SIKs)**. *SLAS Discov* 2017 http://dx.doi.org/10.1177/2472555217717473.

66. Nielsen MMB, Lambertsen KL, Clausen BH, Meyer M, Bhandari DR, Larsen ST, Poulsen SS, Spengler B, Janfelt C, Hansen HS: **Mass spectrometry imaging of biomarker lipids for phagocytosis and signalling during focal cerebral ischaemia**. *Sci Rep* 2016, **6**:39571.

67. Feider CL, Elizondo N, Eberlin LS: **Ambient ionization and FAIMS mass spectrometry for enhanced imaging of multiply charged molecular ions in biological tissues**. *Anal Chem* 2016, **88**:11533-11541.

68. Byron A, Askari JA, Humphries JD, Jacquemet G, Koper EJ, Warwood S, Choi CK, Stroud MJ, Chen CS, Knight D *et al.*: **A proteomic approach reveals integrin activation state-dependent control of microtubule cortical targeting**. *Nat Commun* 2015, **6**:6135.

69. Nicholas KJ, Greenplate AR, Flaherty DK, Matlock BK, Juan JS, Smith RM, Irish JM, Kalams SA: **Multiparameter analysis of stimulated human peripheral blood mononuclear cells: a comparison of mass and fluorescence cytometry**. *Cytometry A* 2016, **89**:271-280.

70. Dey P, Panga V, Raghunathan S, Antoniv T, Falck-Pedersen E, Koch A: **A cytokine signalling network for the regulation of**

inducible nitric oxide synthase expression in rheumatoid arthritis. *PLoS One* 2016, **11**:e0161306.

71. Mrdjen D, Hartmann FJ, Becher B: **High dimensional cytometry of central nervous system leukocytes during neuroinflammation**. *Methods Mol Biol* 2017 http://dx.doi.org/10.1007/978-1-4939-6786-5_22.

72. De Toro J, Herschlik L, Waldner C, Mongini C: **Emerging roles of exosomes in normal and pathological conditions: new insights for diagnosis and therapeutic applications**. *Front Immunol* 2015, **6**:203.

73. Alexander M, Hu R, Runtsch MC, Kagele DA, Mosbruger TL, Tolmachova T, Seabra MC, Round JL, Ward DM, O'Connell RM: **Exosome-delivered microRNAs modulate the inflammatory response to endotoxin**. *Nat Commun* 2015, **6**:7321.

74. Chen WLK, Edington C, Suter E, Yu J, Velazquez JJ, Velazquez JG, Shockley M, Large EM, Venkataramanan R, Hughes DJ *et al.*: **Integrated gut/liver microphysiological systems elucidates inflammatory inter-tissue crosstalk**. *Biotechnol Bioeng* 2017 http://dx.doi.org/10.1002/bit.26370.