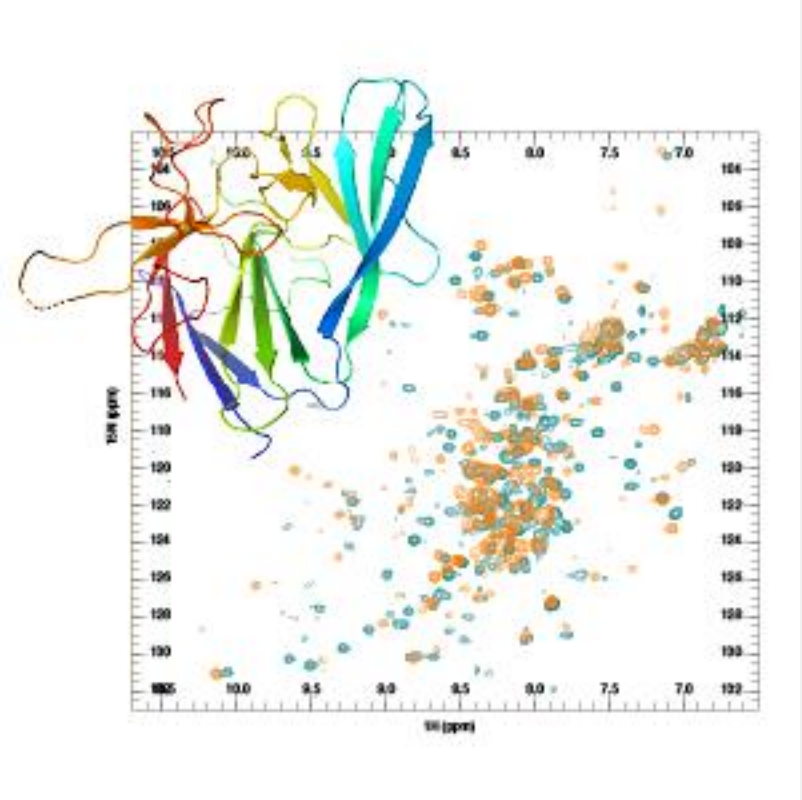
NMR spectroscopy services

NMR SERVICES

NMR spectroscopy services

Broad range of custom protein NMR spectroscopy services

- Protein structure determination in solution
- Peptide secondary structure assessment
- Protein-ligand and protein-protein complex structure determination
- Fragment screening (^{19}F, ligand or protein detected)
- Epitope mapping and dissociation constant (Kd) determinations for compounds
- Higher Order Structure (HOS) validation of antibodies
- Expression and purification of ^{2}H, ^{15}N, and ^{13}C-labelled proteins in E. coli



Ultra High Field NMR Instruments

Access to NMR instruments, automatic assignment, NUS sampling, fragment screening etc.

800 MHz Bruker Avance III HD

- 4 RF channels
- Triple-axis pulsed field gradients
- TXO cryoprobe (1H/13C/15N, 5 mm)
- Triple resonance probe, 8 mm

800 MHz Bruker Avance III HD

- 4 RF channels
- Triple-axis pulsed field gradients
- TCI cryoprobe (1H/13C/15N, 3 mm)
- SampleJet

All instruments at the:
Swedish NMR Centre, Gothenburg

700 MHz Bruker Avance III

- 4 RF channels
- Triple-axis pulsed field gradients
- QCI cryoprobe (1H/19F/13C/15N, 5 mm))
- SampleJet

3x600 MHz Varian Inova/Bruker

- 3-4 RF channels
- Triple-axis pulsed field gradients
- Tripple resonance cryo-probe (1H/13C/15N)
- Triple resonance probe (1H/13C/X)
- 4 mm NANO probe
- Diffusion probe (1H/2H/19F)

900 MHz Bruker Avance III HD

- 4 RF channels
- Triple-axis pulsed field gradients
- TCI cryoprobe (1H/13C/15N, 3 mm)

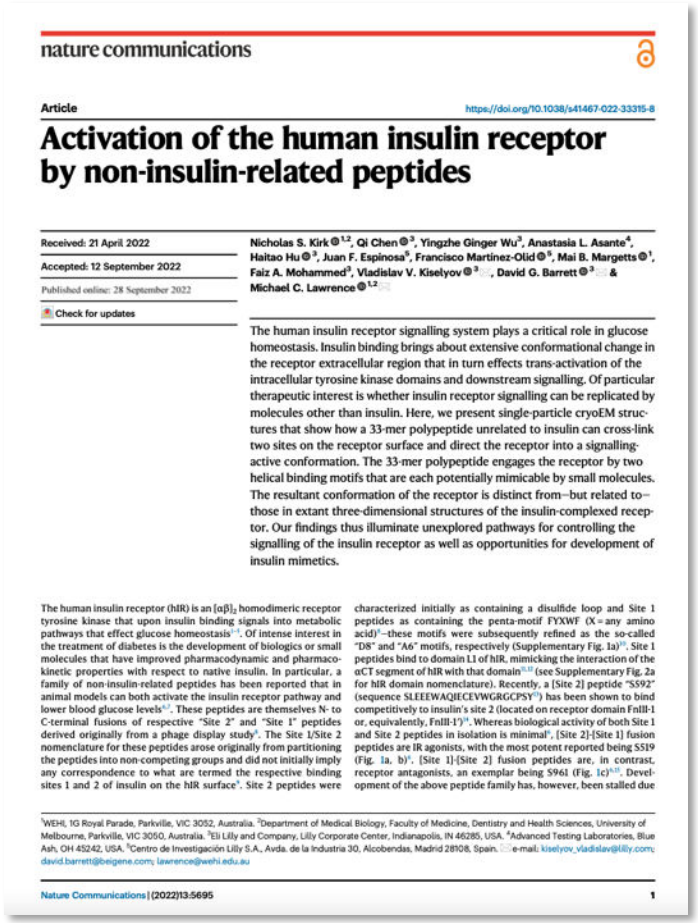
NMR Center at Lund University

- 800 MHz Bruker Avance Neo
- 4 RF channels
- TCI cryoprobe (1H/13C/15N/2H, 5 mm)
- Most sensitive 800 MHz instrument in Scandinavia!

NMR solution structure of an insulin receptor-activating peptide

Client project: NMR structure determination of IM459N21 for Cryo-EM refinement

Collaboration with **Eli Lilly**, Indianapolis, IN



nature communications

Article

https://doi.org/10.1038/s41467-022-33315-8

Activation of the human insulin receptor by non-insulin-related peptides

Received: 21 April 2022

Accepted: 12 September 2022

Published online: 28 September 2022

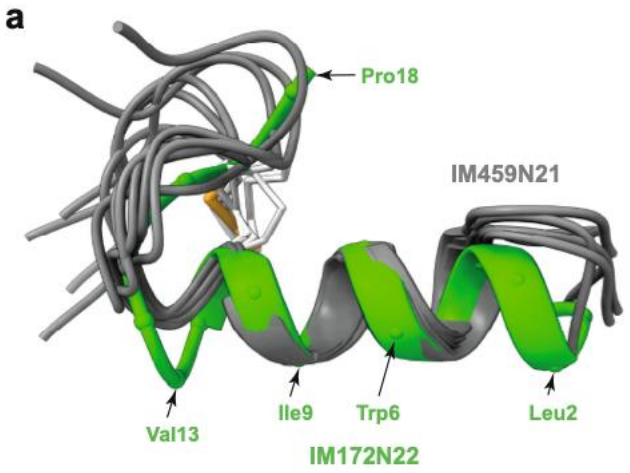
Check for updates

Nicholas S. Kirk[1,2], Qi Chen[3], Yingzhe Ginger Wu[3], Anastasia L. Asante[4], Haitao Hu[3], Juan F. Espinosa[5], Francisco Martinez-Olid[5], Mai B. Margetts[1], Faiz A. Mohammed[3], Vladislav V. Kiselyov[3] ✉, David G. Barrett[3] ✉ & Michael C. Lawrence[1,2] ✉

The human insulin receptor signalling system plays a critical role in glucose homeostasis. Insulin binding brings about extensive conformational change in the receptor extracellular region that in turn effects trans-activation of the intracellular tyrosine kinase domains and downstream signalling. Of particular therapeutic interest is whether insulin receptor signalling can be replicated by molecules other than insulin. Here, we present single-particle cryoEM structures that show how a 33-mer polypeptide unrelated to insulin can cross-link two sites on the receptor surface and direct the receptor into a signalling-active conformation. The 33-mer polypeptide engages the receptor by two helical binding motifs that are each potentially mimicable by small molecules. The resultant conformation of the receptor is distinct from—but related to—those in extant three-dimensional structures of the insulin-complexed receptor. Our findings thus illuminate unexplored pathways for controlling the signalling of the insulin receptor as well as opportunities for development of insulin mimetics.

The human insulin receptor (hIR) is an $[\alpha\beta]_2$ homodimeric receptor tyrosine kinase that upon insulin binding signals into metabolic pathways that effect glucose homeostasis[1–3]. Of intense interest in the treatment of diabetes is the development of biologics or small molecules that have improved pharmacodynamic and pharmacokinetic properties with respect to native insulin. In particular, a family of non-insulin-related peptides has been reported that in animal models can both activate the insulin receptor pathway and lower blood glucose levels[4,5]. These peptides are themselves N- to C-terminal fusions of respective "Site 2" and "Site 1" peptides derived originally from a phage display study[3]. The Site 1/Site 2 nomenclature for these peptides arose originally from partitioning the peptides into non-competing groups and did not initially imply any correspondence to what are termed the respective binding sites 1 and 2 of insulin on the hIR surface[3]. Site 2 peptides were characterized initially as containing a disulfide loop and Site 1 peptides as containing the penta-motif FYXWF (X = any amino acid)[3]—these motifs were subsequently refined as the so-called "D8" and "A6" motifs, respectively (Supplementary Fig. 1a)[6]. Site 1 peptides bind to domain L1 of hIR, mimicking the interaction of the αCT segment of hIR with that domain[7–10] (see Supplementary Fig. 2a for hIR domain nomenclature). Recently, a [Site 2] peptide "S592" (sequence SLEEEWAQIECEVWGRGCPSY[11]) has been shown to bind competitively to insulin's site 2 (located on receptor domain FnIII-1 or, equivalently, FnIII-1')[12]. Whereas biological activity of both Site 1 and Site 2 peptides in isolation is minimal[3], [Site 2]-[Site 1] fusion peptides are IR agonists, with the most potent reported being S519 (Fig. 1a, b)[4]. [Site 1]-[Site 2] fusion peptides are, in contrast, receptor antagonists, an exemplar being S961 (Fig. 1c)[4,13]. Development of the above peptide family has, however, been stalled due

[1]WEHI, 1G Royal Parade, Parkville, VIC 3052, Australia. [2]Department of Medical Biology, Faculty of Medicine, Dentistry and Health Sciences, University of Melbourne, Parkville, VIC 3050, Australia. [3]Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, USA. [4]Advanced Testing Laboratories, Blue Ash, OH 45242, USA. [5]Centro de Investigación Lilly S.A., Avda. de la Industria 30, Alcobendas, Madrid 28108, Spain. ✉e-mail: kiselyov_vladislav@lilly.com; david.barrett@beigene.com; lawrence@wehi.edu.au



NMR solution structure of the IM459N21 peptide

PDB code: 8DI2

Kirk et al., 2022, Nat Commun., 13, 5695.

Acknowledgements

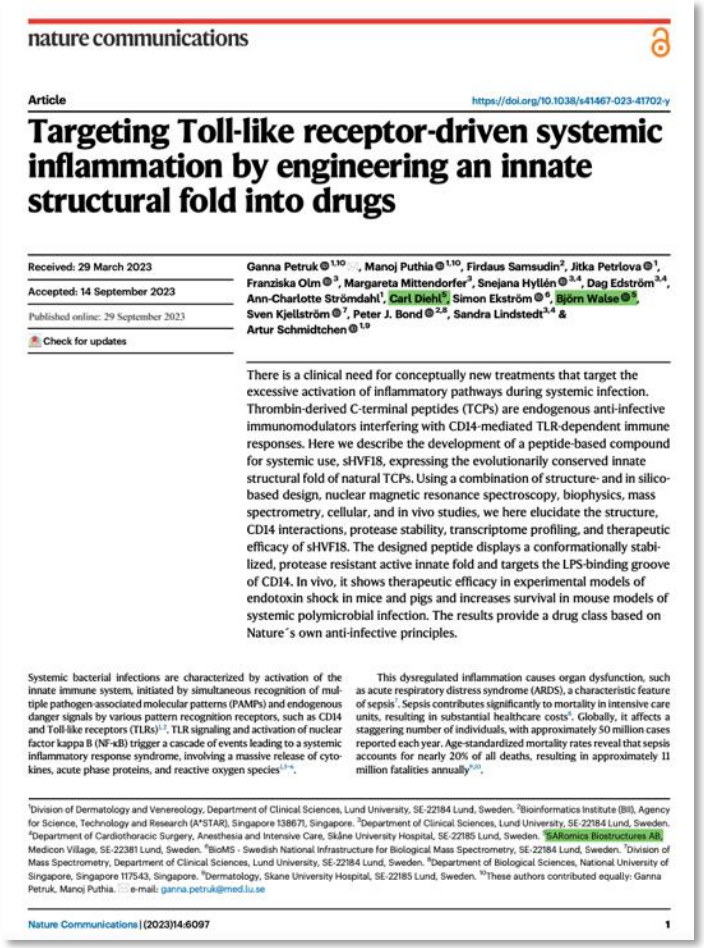
All work undertaken at WEHI by M.C.L., N.S.K. and M.B.M. was funded by a Research Agreement from Eli Lilly and Company (Indianapolis). WEHI receives Victorian State Government Operational Infrastructure Support and funding from the Australian NHMRC Independent Research Institutes Infrastructure Support Scheme. The authors acknowledge the use of instruments at the Monash Ramaciotti Centre for Cryo-Electron Microscopy (a Node of Microscopy Australia) and the use of associated equipment funded by the Australian Research Council grant LE120100090 and thank Hari Venugopal (Monash University) for his assistance in this regard. Part of the NMR data analysis was carried out under contract to SaRomics (Lund, Sweden) and we thank Carl Diehl (SaRomics) for his contribution in this regard. We also thank Julia Cummins and Wayne Hohn (Eli Lilly and Co.) for peptide preparation, and Prof. Ken Siddle (University of Cambridge) for the provision of the hybridoma expressing the monoclonal antibody mAb 83-7.

The findings illuminate unexplored pathways for controlling the signaling of the insulin receptor as well as opportunities for development of insulin mimetics.

NMR solution structure of a stapled anti-inflammatory peptide

Client project: NMR structure determination of sHVF18

Collaboration with **Lund University**



nature communications

Article

https://doi.org/10.1038/s41467-023-41702-y

Targeting Toll-like receptor-driven systemic inflammation by engineering an innate structural fold into drugs

Received: 29 March 2023

Accepted: 14 September 2023

Published online: 29 September 2023

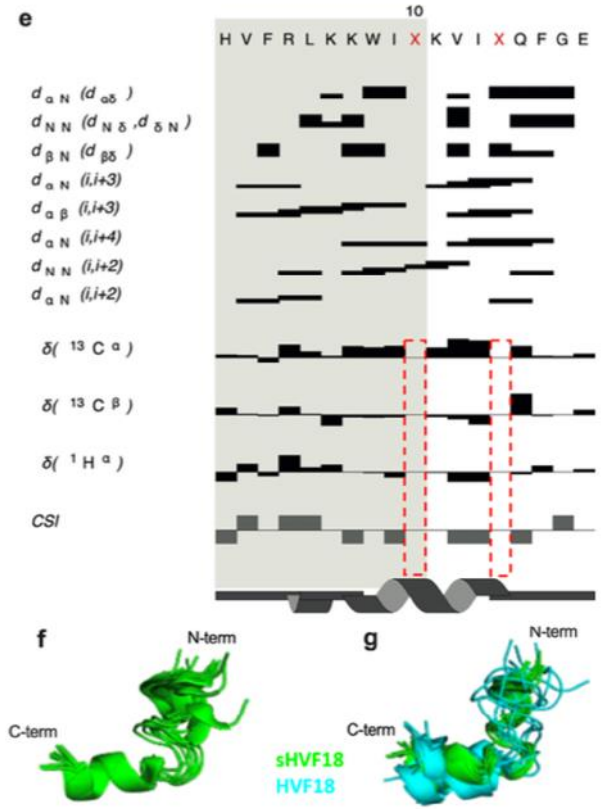
Check for updates

Ganna Petruk[1,10], Manoj Puthia[1,10], Firdaus Samsudin[2], Jitka Petrlova[1], Franziska Olm[3], Margareta Mittendorfer[3], Snejana Hyllén[3,4], Dag Edström[3,4], Ann-Charlotte Strömdahl[1], Carl Diehl[5], Simon Ekström[6], Björn Walse[5], Sven Kjellström[7], Peter J. Bond[2,8], Sandra Lindstedt[3,4] & Artur Schmidtchen[1,9]

There is a clinical need for conceptually new treatments that target the excessive activation of inflammatory pathways during systemic infection. Thrombin-derived C-terminal peptides (TCPs) are endogenous anti-infective immunomodulators interfering with CD14-mediated TLR-dependent immune responses. Here we describe the development of a peptide-based compound for systemic use, sHVF18, expressing the evolutionarily conserved innate structural fold of natural TCPs. Using a combination of structure- and in silico-based design, nuclear magnetic resonance spectroscopy, biophysics, mass spectrometry, cellular, and in vivo studies, we here elucidate the structure, CD14 interactions, protease stability, transcriptome profiling, and therapeutic efficacy of sHVF18. The designed peptide displays a conformationally stabilized, protease resistant active innate fold and targets the LPS-binding groove of CD14. In vivo, it shows therapeutic efficacy in experimental models of endotoxin shock in mice and pigs and increases survival in mouse models of systemic polymicrobial infection. The results provide a drug class based on Nature´s own anti-infective principles.

Systemic bacterial infections are characterized by activation of the innate immune system, initiated by simultaneous recognition of multiple pathogen-associated molecular patterns (PAMPs) and endogenous danger signals by various pattern recognition receptors, such as CD14 and Toll-like receptors (TLRs)[1–3]. TLR signaling and activation of nuclear factor kappa B (NF-κB) trigger a cascade of events leading to a systemic inflammatory response syndrome, involving a massive release of cytokines, acute phase proteins, and reactive oxygen species[4,5]. This dysregulated inflammation causes organ dysfunction, such as acute respiratory distress syndrome (ARDS), a characteristic feature of sepsis[1]. Sepsis contributes significantly to mortality in intensive care units, resulting in substantial healthcare costs[6]. Globally, it affects a staggering number of individuals, with approximately 50 million cases reported each year. Age-standardized mortality rates reveal that sepsis accounts for nearly 20% of all deaths, resulting in approximately 11 million fatalities annually[7,10].

[1]Division of Dermatology and Venereology, Department of Clinical Sciences, Lund University, SE-22184 Lund, Sweden. [2]Bioinformatics Institute (BII), Agency for Science, Technology and Research (A*STAR), Singapore 138671, Singapore. [3]Department of Clinical Sciences, Lund University, SE-22184 Lund, Sweden. [4]Department of Cardiothoracic Surgery, Anesthesia and Intensive Care, Skåne University Hospital, SE-22185 Lund, Sweden. [5]SARomics Biostructures AB, Medicon Village, SE-22381 Lund, Sweden. [6]BioMS - Swedish National Infrastructure for Biological Mass Spectrometry, SE-22184 Lund, Sweden. [7]Division of Mass Spectrometry, Department of Clinical Sciences, Lund University, SE-22184 Lund, Sweden. [8]Department of Biological Sciences, National University of Singapore, Singapore 117543, Singapore. [9]Dermatology, Skane University Hospital, SE-22185 Lund, Sweden. [10]These authors contributed equally: Ganna Petruk, Manoj Puthia. e-mail: ganna.petruk@med.lu.se



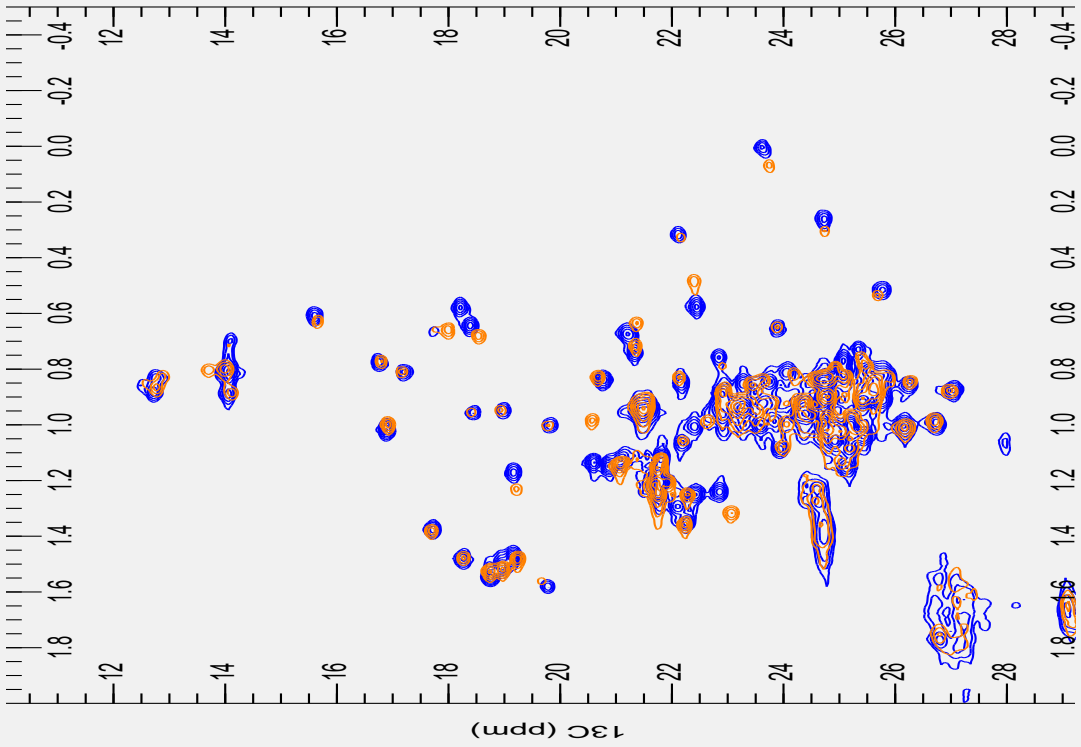
NMR solution structure of the sHVF18 peptide
PDB code: 8BWW

Thrombin-derived C-terminal peptides (TCPs) are endogenous anti-infective immunomodulators interfering with CD14-mediated TLR-dependent immune responses.

- Stapled version, sHVF18, displays a conformationally stabilized, protease resistant active innate fold
- Targets the LPS-binding groove of CD14
- Therapeutic efficacy in experimental models of endotoxin shock *in vivo*
- Increases survival in mouse models of systemic polymicrobial infection.

Petruk et al., 2023, Nat Commun., 14, 6097.

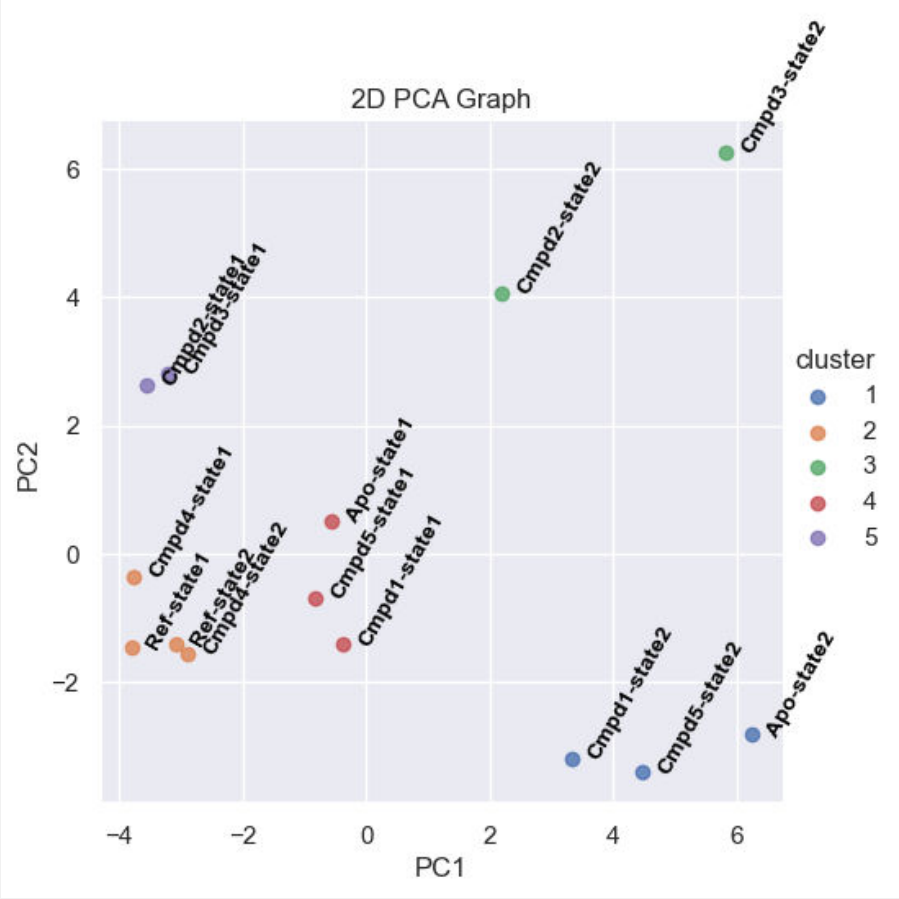
Verification of ligand binding using non-labeled protein



- An array of 2D 13C-HSQC spectra of a protein with addition of ligand
- Cross peaks from residues affected by the interaction are shifted

- No labelling required
- Size limit up to 150 kDa
- Quick and cost efficient
- Requires backbone assignment if binding site mapping is wanted

Binding mode identification using PCA and cluster analysis

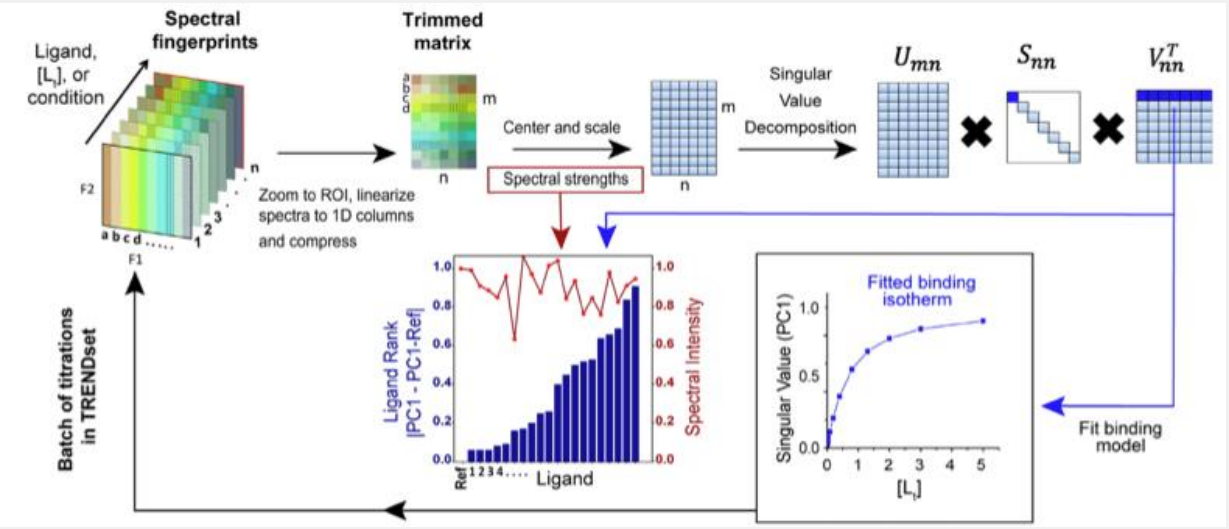


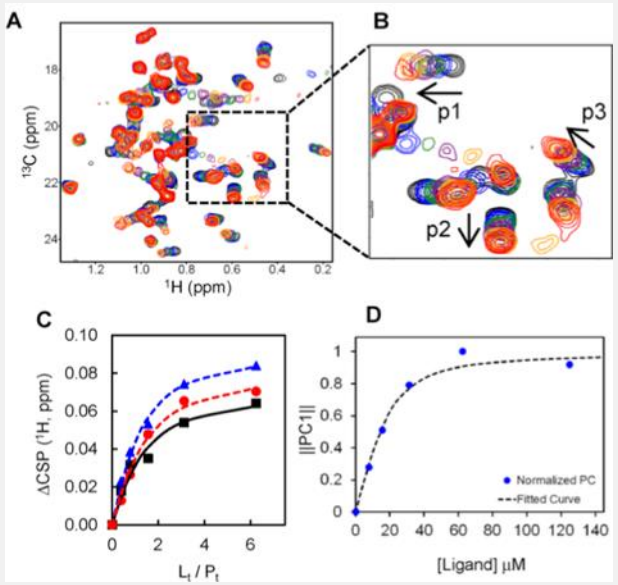
Analysis of binding modes of cmpds 1-5 for two different protein states
Cmpd 4 has a similar binding mode to a reference cmpd (Ref) for both protein states (cluster 2)

- 2D spectra are linearized and principal component analysis followed by clustering is applied
- Compounds with similar effect on 2D spectra can be clustered to characterize binding modes

- No assignment is required
- 2D ^{15}N or ^{13}C spectra can be used (but is not required)
- 2D ^{13}C HSQC spectra of non-labelled protein
- Small differences in spectra can be detected
- Large amount of data can easily be processed

Fragment ranking and Kd determination using PCA and cluster analysis





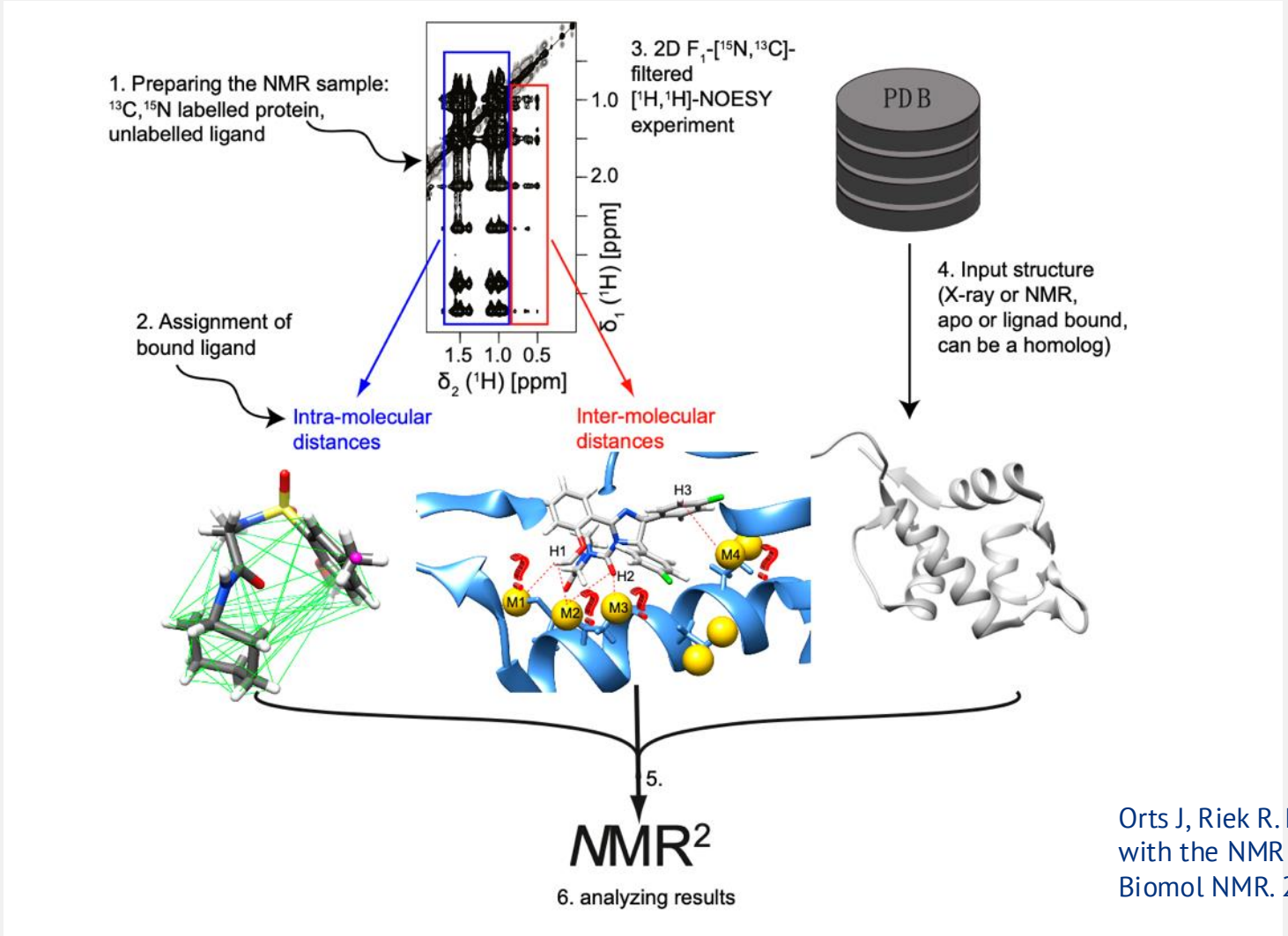
- 2D spectra are linearized and principal component analysis is applied
- No visual analysis required
- Fragments are ranked based on first principal component (PC1)
- Kd can be determined using concentration dependence for PC1

- No assignment is required
- 2D ^{15}N or ^{13}C spectra can be used
- 2D ^{13}C HSQC spectra of non-labelled protein
- Small differences in spectra can be detected
- Large amount of data can easily be processed

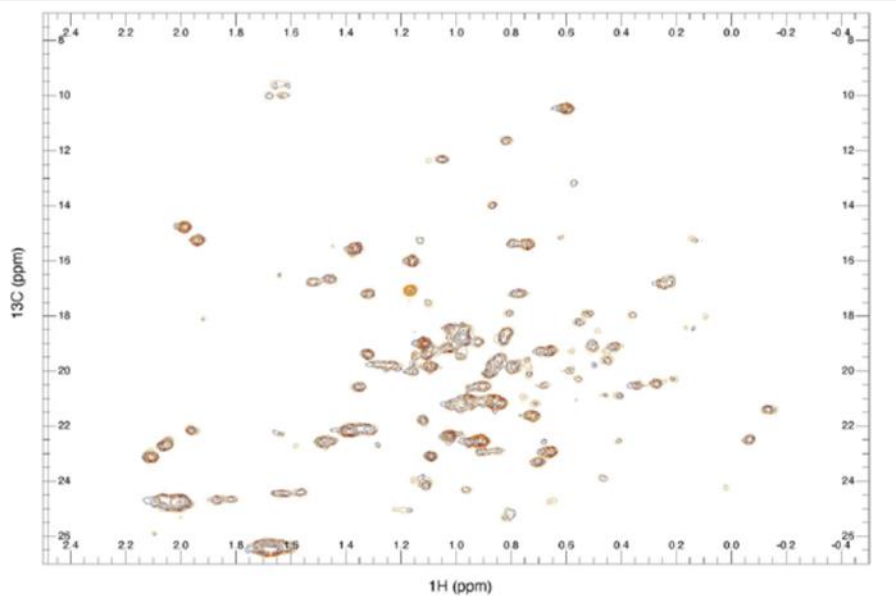
Namanja, Andrew T., Jia Xu, Haihong Wu, Qi Sun, Anup K. Upadhyay, Chaohong Sun, Steven R. Van Doren, and Andrew M. Petros. 2019. "NMR-Based Fragment Screening and Lead Discovery Accelerated by Principal Component Analysis." *Journal of Biomolecular NMR* 73 (12): 675–85. https://doi.org/10.1007/s10858-019-00279-9.

NMR² method implemented

Enables fast structure determination of bound ligand



Orts J, Riek R. Protein-ligand structure determination with the NMR molecular replacement tool, NMR². J Biomol NMR. 2020 Nov;74(10-11):633-642.

NMR Higher Order Structure (HOS)

- 2D NMR spectroscopy can be used to study and compare HOS of biologics using unlabeled proteins
- Proteins in formulation buffer can be studied with little need for sample preparation
- Effects on HOS of oxidation or surfactant concentration can be studied
- Time resolved processes can be followed using 2D spectra with <1 h / spectra
- Applications:
  - Batch-to-batch consistency
  - Biosimilar/originator comparability
  - Formulation optimization