

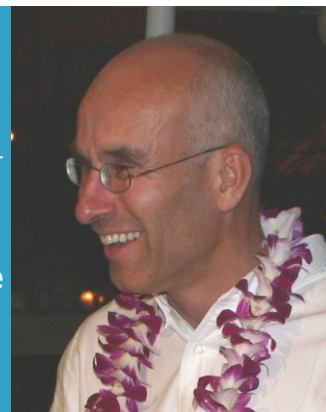
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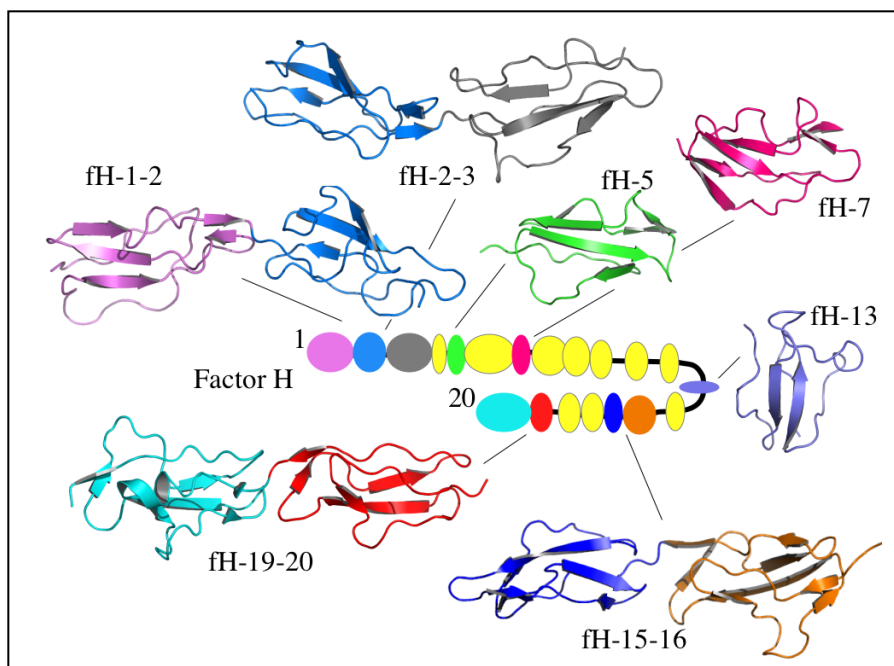
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Research Interests: protein structure, nuclear magnetic resonance spectroscopy, immunology, complement system



Our current focus is on the 30 or so proteins of the human complement system. These proteins work together in an orchestrated fashion to attack invaders, release mediators of inflammation and rid the body of foreign cells and viruses, immune complexes and apoptotic or necrotic cells. The complement system includes both an amplification cascade and a positive feedback loop. Thus there is the potential for damage to host tissues. Indeed numerous autoimmune, degenerative and inflammatory diseases involve inappropriate or disproportionate activation of complement. There is strong interest in the potential for therapies that modulate complement in the body. Rational design of therapies requires in depth knowledge of mechanism.

Despite decades of research, we have little idea of how most of the proteins in the complement system actually work. Progress has been held back by a lack of detailed structural insights. In recent years, however, major progress has been made towards an atomic resolution understanding of this set of proteins and their interactions with one another. For example, our work on the complement regulatory protein factor H, from which we have solved structures of individual domains and domain-pairs, is summarised below. This "divide-and-conquer" approach is necessary given the size, flexibility and multiple-domain nature of most of the complement proteins.



We have optimised protocols for recombinant expression of complement proteins in the yeast vector *Pichia pastoris*. We have excellent facilities for molecular biology, protein purification (we are part of the Edinburgh Protein Production Facility), biophysical characterisation and NMR (including an 800 MHz spectrometer with cryoprobe). We are increasingly collaborating with crystallographers and adding new biophysical approaches to our armory of techniques. In particular we have been incorporating tags into our recombinant proteins with the aim of using fluorescence resonance energy transfer and electron paramagnetic resonance spectroscopy. These techniques will provide long-range distance information that will be combined with the more local structural afforded by high-resolution NMR studies. Thus we will be in a position to examine the overall architecture as well as the detailed structure of these proteins and their complexes.

REPRESENTATIVE RECENT PUBLICATIONS

1. Pechtl IC, Neely RK, Dryden DT, Jones AC, Barlow PN Use of time-resolved FRET to validate crystal structure of complement regulatory complex between C3b and factor H (N terminus) *Protein Science* 2011 [Epub ahead of print] PubMed PMID: 21936007.
2. Maciejewski M, Tjandra N, Barlow PN Estimation of interdomain flexibility of N-terminus of factor H using residual dipolar coupling *Biochemistry* 2011 **50**(38):8138-49. PubMed PMID: 21793561.
3. Pechtl IC, Kavanagh D, McIntosh N, Harris CL, Barlow PN Disease-associated N-terminal complement factor H mutations perturb cofactor and decay-accelerating activities *J Biol Chem* 2011 **286**(13):11082-90. PubMed PMID: 21270465.
4. Morgan HP, Schmidt CQ, Guariento M, Blaum BS, Gillespie D, Herbert AP, Kavanagh D, Mertens H, Svergun DI, Johansson CM, Uhrin D, Barlow PN, Hannan JP Structural basis for engagement by complement factor H of C3b on a self surface *Nature Struct Mol Biol* 2011 **18**(4):463-70. PubMed PMID: 21317894.
5. Schmidt CQ, Slingsby FC, Richards A, Barlow PN Production of biologically active complement factor H in therapeutically useful quantities *Protein Expr Purif* 2011 **76**(2):254-63. PubMed PMID: 21146613.