

Impact case study (REF3b)

Institution: University College London / Birkbeck College
Unit of Assessment: 5 - Biological Sciences
Title of case study: Use of the structure of serum amyloid P, a modulator of amyloid formation, for drug discovery and development
1. Summary of the impact (indicative maximum 100 words) <p>Serum amyloid P, or pentraxin-2, is a pentameric calcium-binding protein that binds to amyloid fibrils. It has been implicated in the protection of those fibrils from proteolytic digestion and in the immune response to tissue damage. The structure of pentraxin-2 was first solved by Steve Wood and his co-workers in Tom Blundell's lab at Birkbeck in the 1990s. Wood has continued his work on the pentraxins at UCL, and the company Pentraxin Therapeutics Ltd was spun out of UCL to design and develop pentraxin-binding ligands (based on its structure) as potential treatments for Alzheimer's disease and amyloidosis. Promedior Inc. in the US is developing recombinant forms of pentraxin to control fibrosis. Several of these molecules are now in clinical trials.</p>
2. Underpinning research (indicative maximum 500 words) <p>Deposits of a fibrillar form of protein known as amyloid have been associated with a wide range of human diseases including Alzheimer's disease, the most common form of dementia. Amyloid fibres formed from different proteins have essentially the same "cross-beta" structure. A number of other proteins have been discovered to be associated with amyloid fibres, among them a protein called serum amyloid P (SAP). This protein has been shown to not be required for fibre formation <i>in vitro</i> but is now known to stabilise the fibres <i>in vivo</i>.</p> <p>Knowledge of the three-dimensional structure of this protein has been an essential pre-requisite to understanding the mechanism of its interaction with amyloid. The structure of the native, pentameric form of SAP was first determined by a group led by Steve Wood in the former Department of Crystallography at Birkbeck, working under the overall direction of Professor (now Sir) Tom Blundell. This work was published in <i>Nature</i> in 1994 [1] and the structure released by the Protein Bank with the code 1SAC. The paper had attracted 336 citations by August 2013.</p> <p>Serum amyloid P is known to bind to amyloid fibres; to other protein ligands including fibronectin and glycosaminoglycans; and to DNA in a calcium dependent manner. This 3D structure, the first to be determined for a pentraxin, revealed that each SAP monomer has a fold remarkably similar to that seen in a family of carbohydrate-binding proteins, the legume lectins. Binding positions were identified in the crystal structure for two calcium ions, largely coordinated by the side chains of negatively charged amino acids that are conserved throughout the SAP family. SAP is known to be remarkably resistant to protease digestion in the presence of calcium, and this may be partly responsible for the protease resistance of amyloid fibrils <i>in vivo</i>.</p> <p>Following the determination of the crystal structure of SAP, the group undertook a comparative analysis of the pentraxin family, comparing the sequences, tertiary structures and quaternary arrangements of SAP with human C-reactive protein (CRP), Syrian hamster SAP and <i>Limulus polyphemus</i> CRP. Calcium-mediated ligand binding by each of these proteins was found to be similar, but sequence differences in the hydrophobic pocket explained the differential ligand specificities exhibited by the homologous proteins [2].</p> <p>Work by Wood and Dr Erhard Hohenester, a postdoctoral fellow at Birkbeck, to solve the structure of a decameric complex of SAP with a bound ligand, 2'-deoxyadenosine-5'-mono-phosphate (dAMP), was undertaken at Birkbeck (and written up and published after Wood left in 1996) [3]. This novel structure for the SAP-ligand complex showed the calcium sites shielded against competing ligands and illustrated that the modest affinity of this protein for small ligands could be enhanced by cooperativity between the subunits of a complex. This cooperativity in ligand binding</p>

Impact case study (REF3b)

was utilised in the discovery of the candidate drug molecule CPHPC, discussed below.

Wood and his co-workers originally speculated that knowledge of the binding mode of SAP to calcium and to carbohydrate- and phosphate-containing compounds might lead to the development of drugs to treat amyloidosis by modulating SAP binding to amyloid and hence amyloid's proteolytic digestion. In work started at Birkbeck, his group later published the structure of SAP bound to a carbohydrate ligand, confirming many of these speculations [4].

Wood has continued his productive work on amyloid proteins after leaving Birkbeck, first at Southampton and then at UCL. He is now a Professor in the Centre for Amyloidosis and Acute Phase Proteins, Division of Medicine, University College London, and he continues to collaborate with his former colleagues at Birkbeck as a core member of the ISMB. His current work builds upon the work described above, including targeting the related PTX-1C-reactive protein for new treatments for myocardial infarction and stroke.

3. References to the research (indicative maximum of six references)

- [1] Emsley J, White HE, O'Hara BP, Oliva G, Srinivasan N, Tickle IJ, Blundell TL, Pepys MB, Wood SP. Structure of pentameric human serum amyloid P component. *Nature*. 1994 Jan 27;367(6461):338-45. <http://dx.doi.org/10.1038/367338a0>
- [2] Srinivasan N, White HE, Emsley J, Wood SP, Pepys MB, Blundell TL. Comparative analyses of pentraxins: implications for protomer assembly and ligand binding. *Structure*. 1994 Nov 15;2(11):1017-27. [http://dx.doi.org/10.1016/S0969-2126\(94\)00105-7](http://dx.doi.org/10.1016/S0969-2126(94)00105-7)
- [3] Hohenester E, Hutchinson WL, Pepys MB, Wood SP. Crystal structure of a decameric complex of human serum amyloid P component with bound dAMP. *J Mol Biol*. 1997 Jun 20;269(4):570-8. <http://dx.doi.org/10.1006/jmbi.1997.1075>
- [4] Thompson D, Pepys MB, Tickle I, Wood S. The structures of crystalline complexes of human serum amyloid P component with its carbohydrate ligand, the cyclic pyruvate acetal of galactose. *J Mol Biol*. 2002 Jul 26;320(5):1081-6. [http://dx.doi.org/10.1016/S0022-2836\(02\)00514-4](http://dx.doi.org/10.1016/S0022-2836(02)00514-4)

Funding

MRC. Wood S, Tickle I, Pepys M. Structure and ligand binding of serum amyloid P component. 1995-8. £126,331

Wood SP, Pepys MB. Structural analysis of ligand recognition and associated biological roles of pentraxins. 2000-3. £227,474

4. Details of the impact (indicative maximum 750 words)

The first therapeutic applications of modulating the interaction of serum amyloid P with its ligands revolved around compounds that interact with it to remove it from pathogenic amyloid fibres and therefore render those fibres more vulnerable to proteolytic degradation. A lead compound, CPHPC, was developed and shown to cross-link two SAP pentamers, removing the protein both from circulation and from interaction with amyloid. The discovery of this compound was critically dependent on the insights into cooperative ligand binding discovered through the decamericSAP/dAMP structure solved in Wood's lab at Birkbeck and reported in reference [2] above.

A biotech company, Pentraxin Therapeutics Ltd (PTL), was spun out of UCL in 2001 to design drugs for the treatment of amyloid-related diseases and develop them; serum amyloid P became its first validated target [a], resulting in a series of patents. For example, in 2008, formulations of CPHPC were patented as a possible treatment for Alzheimer's disease [b]. Preliminary results (published by the Centre for Amyloidosis and Acute Phase Proteins) showed SAP depletion *in vivo*

Impact case study (REF3b)

[c]; however despite these and other promising results in mouse models and human data showing that CPHPC causes a fall in circulating concentrations of SAP, this drug has made little progress in clinical trials as a monotherapy for Alzheimer's disease.

Wood and his co-workers in the Centre for Amyloidosis and Acute Phase Proteins at UCL and at PTL have now licensed a combination of CPHPC and anti-SAP antibodies to GlaxoSmithKline (GSK) as a treatment for systemic amyloidosis [d]. This therapy involves administering CPHPC (i/d GSK2315698) first to remove SAP from the patients' circulation and then administering an anti-SAP antibody (i/d GSK2398852); with no SAP in circulation, this antibody should be directed to amyloid-bound SAP to activate the patients' immune systems and destroy the amyloid.

The first Phase I clinical trial of the combination therapy began recruiting patients diagnosed with systemic amyloidosis in June 2013 [e]. This is a two-part trial; the first part is a dose escalation study to determine the safety of the two components and the second will assess its dose-response and effect on SAP concentrations in more detail. It is co-sponsored by three London hospitals and so contributes to the continuation of skilled employment in the UK, both at GSK and at these sites. GSK have hailed the importance of their collaboration with the Centre for Amyloidosis and Acute Phase Proteins as part of their strategy of academic engagement in drug discovery [d, f]. The potential value of this combination therapy has already attracted the attention of patient groups, despite its early stage of development; Amyloidosis Australia has described it as "...the most wonderful news about Amyloidosis we had heard in the past 8 years" [g].

The known function of circulating SAP as a mediator of the inflammatory response is being exploited by the innovative biotech company Promedior Inc., based in Lexington, MA, USA. Promedior's work focuses on the development of recombinant forms of SAP (Pentraxin-2 or PTX-2) as treatments for fibrosis. This term refers to the formation of excessive connective tissue in response to inflammation, a response that plays an important part in many types of chronic organ failure. The mechanism for the activity of PTX-2 against fibrosis is based on its specific, calcium-mediated recognition of so-called "damage-mediated molecular patterns" (DAMPs) which are exposed when cells are damaged. This promotes the removal of the damaged proteins by immunoglobulins on the surface of macrophages. The mechanism of this interaction was elucidated using recent crystallographic data obtained by Promedior, which shows the calcium- and immunoglobulin-binding sites lying on opposite sides of the pentraxin complex. These studies, also, would not have been possible without access to the initial structures obtained by Wood's group at Birkbeck from 1994 onwards.

The most advanced compound in the Promedior pipeline is currently (summer 2013) an injected form of PTX-2 designated PRM-151 [h]. This is in early clinical trials for the treatment of idiopathic pulmonary fibrosis (IPF), a chronic, progressive lung disease that kills about 5,000 people annually in the UK. The FDA and EMA have designated PRM-151 as an "orphan drug" for this condition, giving Promedior some specific commercial and marketing incentives [i]. A Phase I trial in IPF patients and healthy volunteers has shown that PTX-2 is safe, suggested that it can reduce fibrocyte levels in IPF patients, and recommended it for further clinical trials [j, k]; a Phase 2 trial is now in progress.

Pentraxin formulations are also being developed for fibrous diseases of the retina. A Phase 2 clinical trial of PRM-151 to prevent scarring following trabeculectomy in glaucoma enrolled 124 patients from 14 European study centres and was completed in November 2012 but is not yet published. A variant form of recombinant SAP, PTX-169, has now been developed for injection into the eye. Pre-clinical results presented at the Association for Research in Vision and Ophthalmology in 2012 indicate that this compound shows promise in diabetic retinopathy and age-related macular degeneration, each of which affects millions of people worldwide [l]; clinical trials are planned. Promedior is a thriving company and its SAP-based programmes make a significant contribution to economic growth and skilled employment in Massachusetts.

5. Sources to corroborate the impact (indicative maximum of 10 references)

Impact case study (REF3b)

- [a] Corroboration of the establishment of Pentraxin Therapeutics Ltd, its patent holdings and licensing agreements can be obtained from the Managing Director, UCL Business PLC. Contract details provided. <http://pentraxin.wordpress.com/rd-programs/>
- [b] Patent information for CPHPC as a potential treatment for Alzheimer's disease http://worldwide.espacenet.com/publicationDetails/biblio?CC=WO&NR=2008014232&KC=&FT=E&locale=en_EP
- [c] Kolstoe SE, Ridha BH, Bellotti V, Wang N, Robinson CV, Crutch SJ, Keir G, Kukkastenvehmas R, Gallimore JR, Hutchinson WL, Hawkins PN, Wood SP, Rossor MN, Pepys MB. Molecular dissection of Alzheimer's disease neuropathology by depletion of serum amyloid P component. Proc Natl Acad Sci U S A. 2009 May 5;106(18):7619-23. <http://dx.doi.org/10.1073/pnas.0902640106>
- [d] Press release regarding licensing to GSK: <http://www.uclb.com/news-and-events/news-post/ucl-and-gsk-join-forces-to-develop-combined-small-molecule-antibody-treatment-for-rare-disease>
Press coverage: www.europharmatoday.com/2009/03/gsk-vallance-teams-up-with-exuniversity-london-colleagues-in-pentraxin-deal.html
- [e] Phase I clinical trial of a combination of CPHPC and an anti-SAP antibody: <http://clinicaltrials.gov/ct2/show/NCT01777243>
- [f] FT coverage: "GSK looks to academia for new drugs" <http://www.ft.com/cms/s/0/d1e31184-37a5-11e0-b91a-00144feabdc0.html>
- [g] www.amyloidosis.com.au/index.php?option=com_content&view=article&id=77&Itemid=41
- [h] Promedior pipeline showing the development of recombinant human serum amyloid P/pentraxin 2 (PRM-151) for fibrosis related conditions: <http://www.promedior.com/pipeline/pipeline.html>
- [i] Orphan drug designation for PRM-151 in IPF: <http://www.businesswire.com/news/home/20120307006426/en/Promedior-Receives-U.S.-Orphan-Drug-Designation-PRM-151>
- [j] Dillingh MR, van den Blink B, Moerland M, van Dongen MG, Levi M, Kleinjan A, Wijsenbeek MS, Luper ML Jr, Harper DM, Getsy JA, Hoogsteden HC, Burggraaf J. Recombinant human serum amyloid P in healthy volunteers and patients with pulmonary fibrosis. Pulm Pharmacol Ther. 2013 Feb 4. pii: S1094-5539(13)00054-0. <http://dx.doi.org/10.1016/j.pupt.2013.01.008>.
- [k] Abstract # A5707 at May 2013 conference of the American Thoracic Society, available from <https://cms.psav.com/cPaper2013/ats2013/myitinerary>
Press release: <http://www.promedior.com/news/releases/2013%200522%20Promedior%20PRM-151%20IBS%20Ph1b%20Results.html>
- [l] Formica R, Shen J, Seidel CP, Hackett SF, Kramer MS, Luper ML, Campochiaro PA(2012). Recombinant Pentraxin-2 (rPTX-2) Shifts Macrophage Phenotype, Suppresses Subretinal NV, and Reduces Associated Vascular Leakage and Collagen Deposition. Invest Ophthalmol Vis Sci 2012(53): E-Abstract 1211. <http://bit.ly/17h7zbn>