

Why does the A β peptide of Alzheimer share structural similarity with antimicrobial peptides?

Annalisa Pastore^{1,2}, Francesco Raimondi³, Lawrence Rajendran^{1,2} & Piero Andrea Temussi^{1,2,4}

The A β peptides causally associated with Alzheimer disease have been seen as seemingly purposeless species produced by intramembrane cleavage under both physiological and pathological conditions. However, it has been increasingly suggested that they could instead constitute an ancient, highly conserved effector component of our innate immune system, dedicated to protecting the brain against microbial attacks. In this antimicrobial protection hypothesis, A β aggregation would switch from an abnormal stochastic event to a dysregulated innate immune response. In this perspective, we approach the problem from a different and complementary perspective by comparing the structure and sequence of A β (1-42) with those of bona fide antimicrobial peptides. We demonstrate that A β (1-42) bears convincing structural similarities with both viral fusion domains and antimicrobial peptides, as well as sequence similarities with a specific family of bacterial bacteriocins. We suggest a model of the mechanism by which A β peptides could elicit the immune response against microbes.

A β peptides are at the root of the pathology of Alzheimer disease (AD)¹, one of the devastating diseases of our increasingly ageing society. The peptides originate from the action of specific proteases, called secretases, on the amyloid precursor protein (APP) inside the membrane of neuronal cells. Sequential cleavage by β -(BACE) and γ -secretase produces different peptides ranging from 1-37 to 1-43 amyloid- β peptide fragments, with 1-40 being the most abundant and 1-42 being the most aggregation prone and more toxic. AD mostly affects people over 65 years of age, but the non-symptomatic phase might last several decades, during which the peptides form large aggregates (plaques) which contain amyloid fibrils of A β peptides².

The aggregates first observed by Alois Alzheimer in 1906 were originally regarded as the culprits of AD leading to what is now commonly called the amyloid hypothesis¹. The traditional formulation of the amyloid hypothesis points to the cytotoxicity of mature aggregated amyloid fibrils, which are believed to be the toxic form of the protein responsible for disrupting the

¹UK-Dementia Research Institute (UK-DRI) at King's College London, London, UK. ²The Maurice Wohl Institute of King's College London, 5 Cutcombe Road, SE5 9RT London, UK. ³Scuola Normale Superiore, Piazza dei Cavalieri, Pisa, Italy. ⁴Dipartimento di Scienze Chimiche, Università di Napoli Federico II, Napoli, Italy. ✉email: annalisa.pastore@crick.ac.uk; temussi@unina.it

cellular calcium ion homeostasis, thus inducing apoptosis. After the original upraising of the amyloid hypothesis, however, it started to become progressively clear that toxicity of the final aggregates was at most marginal: fibrils could be absent or sparse in advanced AD patients or dense in mild patients or healthy individuals. The culprit changed, and the main cause of cell damage was attributed to A β oligomers or, more likely, to an ensemble of oligomers of different stoichiometries and/or morphologies formed during the process of aggregation³. A drastically different alternative views A β monomers as potential agents capable of damaging membranes. Indeed, there is a vast literature that has studied the effects of A β peptides on the membranes and how they affect their properties⁴.

Almost two decades ago, we suggested that A β might function as a viral peptide⁵ based on sequence and structure similarities with the influenza virus fusion domain⁶, hinting at a pore-formation (hereafter referred to as poration) mechanism of interaction⁷. Shortly after, the structure of the influenza virus fusion domain allowed suggestion of a detailed model for the membrane poration mechanism⁸. It could be easily foreseen that the ability to perforate membranes could be used against own brain cells but also against invasive cells, a feature reminiscent not only of viruses but, more in general, of antimicrobial peptides (AMPs).

The “viral-like nature hypothesis” was not followed up by others, but during the last 9 years it was repeatedly suggested that A β peptides may act as AMPs⁹. Both this suggestion and the viral hypothesis open a completely different perspective to the role of A β and directly links neurodegeneration to immunology. Under this hypothesis, A β would not be a peptide released into the cell by accident or genetic predisposition, but a specific and generalised response to foreign agents¹⁰. This “Antimicrobial Protection Hypothesis” would thus significantly change the paradigm, and identify A β and its aggregation as the extreme conclusion of a long chain of events most of which would be a natural response of our immunological system to intruders. Under this hypothesis, A β would, in other words, return to the stage playing an essential element of the neuronal well-being rather than being a foe.

Here, we revisit the membrane poration hypothesis of A β and propose new important aspects that could support the AMP hypothesis: we focus on a completely underemphasised

perspective that compares the sequence and structural aspects of A β peptides with those of other AMPs. We do hope that this work will be inspirational to other researchers and suggest new avenues to approach the role of A β in AD.

An immunologic point of view of AD. The idea that infection could play a relevant role in the pathogenesis of AD was already proposed in the early years of AD research, but abandoned soon after¹¹. The first paper showing experimental evidence in favour of an antimicrobial activity of A β was published in 2010¹² demonstrating that A β peptides have an efficacy comparable to that of the well-known human AMP LL-37. Early studies on A β as an AMP were summarised in an insightful review¹³, where the authors stated that the data published after 2010^{13–15} showed convincingly that A β peptides can indeed act as AMPs, killing clinically relevant microbes. The paradigm of A β as an AMP was taken a step forward by Fulop et al.¹⁶, who proposed explicitly that A β may act as a natural defence against infections which becomes a menace only when the inflammation becomes chronic. The fact that A β behaves as an AMP adds credence to an infection origin in the aetiology of AD. It was independently noticed that transgenic mice raised in conventional husbandry develop neurodegeneration more quickly than if raised in pathogen free conditions¹⁷. While this observation could simply be explained as the consequence of better conditions, it could be suggestive, together with other evidence of a correlation between infection and disease. Different groups were able to relate the defence mechanism to aspects of the link between infection and senescence and demonstrated an increase in bacterial populations in Alzheimer brain tissue compared with normal¹⁸. The discovery of pathogens in AD patients’ brains hints at the emergence of a link between microbiota and senescence¹⁹. Gingipain, a *Porphyromonas gingivalis* toxin, was detected not only in the brains of people deceased by AD but also in the brains of old people who died prior to developing AD²⁰.

AMPs and their structure. AMPs, as crucial components of the innate immune system, are able to kill a variety of microbes, including bacteria, viruses, fungi and yeasts. The first reported AMPs were probably those described by Zeya and Spitznagel as a family of low-molecular-weight cationic peptides with selective antimicrobial activity^{20–22}.

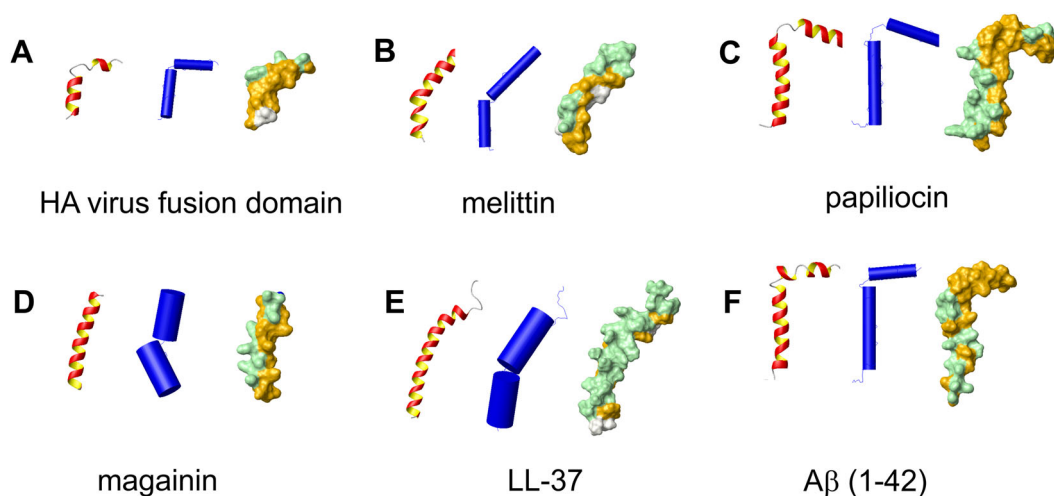


Fig. 1 Comparison of the structures of A β (1–42) with those of selected helical antimicrobial peptides. All structures are represented left to right as ribbon, schematic secondary structure in which helices are depicted as cylinders and a surface picture enhancing the distribution of polar (green) and apolar (orange) residues. **a** HA virus fusion domain (1ibn); **b** melittin (2mlt); **c** papiliocin (2la2); **d** magainin (2mag); **e** LL-37 (2k6o) and **f** A β (1–42) determined by NMR in an aqueous mixture of hexafluoroisopropanol (pdb id 1iyt)⁵. Structures were generated using MOLMOL⁵².

cystatin superfamily of proteins. Cathelicidins act as precursor molecules that, after proteolysis, release a linear AMP. The most important human AMP derived from cathelicidin is LL-37, a highly basic amphipathic peptide of 37 residues with 11 basic and 13 strongly apolar residues. The solution structure³⁶ consists of two helices spanning residues 2–31, with a kink between residues 14 and 16 (Fig. 1e).

Structural comparison with A β (1–42). If we now compare these peptides with A β (1–42), we find interesting similarities. Among the several structures available, we selected that (pdb 1iyt)⁵ in a mixed (20/80) water/hexafluoroisopropanol solution, which is a medium that mimics the membrane environment³⁷. The structure consists of two helices at an angle close to 90° and encompassing residues 8–25 and 28–38, with the connecting link adopting a regular type I β -turn (Fig. 1f). Among the AMPs analysed here, the structure most similar to A β is that of papi-liocin: the tilt angle between the helices is similar and the surfaces, particularly in the C-terminus, have a similar distribution of apolar residues. The structure of the influenza virus fusion domain is also similar to the C-terminus of A β (1–42)⁵. In particular, the angle between the helices is almost identical and the distribution of residues makes the concave surface of both structures apolar.

Exploring the sequence space. We then explored dbAMP (<http://140.138.77.240/~dbamp/index.php>, v1.4), a comprehensive meta-database that collects peptides with reported antimicrobial activity, to search for sequence similarities with A β (1–42). Searches were performed by PSIBLAST³⁸, using ten iterations, turning off composition-based statistics and filtering out low complexity regions. This revealed ten sequences with significant matches (e -value < 10) to A β (1–42) (Fig. 2; Supplementary Table 1a), half of which are experimentally validated AMPs. Notably, also A β (1–42) and A β (1–40) are reported in dbAMP as validated AMPs. Three validated AMPs gave significant matches to A β (1–42): the Bacteriocin carnobacteriocin BM1 (dbAMP_00283, gene: *cbnBM1*, Uniprot ID: CBB1_CARML) and two other bacteriocines not yet reviewed in Swissprot

(dbAMP_01287, gene: *blp1a*, Uniprot ID: I0B595_9LACO and dbAMP_06900, gene: BACERE00183_06588, Uniprot ID: A0A1N7URV8_BACCE) (Supplementary Table 1a). Multiple alignment of these sequences revealed conserved patterns flanking the region that hosts the kink in A β (1–42). We identified an invariant sequence signature (i.e., GXXXGG, where X is an apolar amino acid in most of the sequences including A β (1–42)) at the peptide C-terminus, and a conserved motif (i.e., XXXXXG) N-terminally preceding the kink. Interestingly, the corresponding peptides of close APP homologues such as APLP1/2, which are characterised by an incomplete GXXXGG motif (Fig. 2a), are not reported by all prediction programmes as having antimicrobial activity.

As an additional and independent test, we inspected the sequence similarity of A β (1–42) with peptides from dbAMP which were experimentally validated irrespective of PSIBLAST similarity searches. We restricted the analysis to sequences having generic features similar to those of A β (1–42), i.e., being shorter than 50 amino acids in length, having less than four cysteines (to avoid considering AMPs containing β -hairpins) and matching to Swissprot protein regions annotated as having a transmembrane topology or being localised within the membrane (Supplementary Table 1b). This led to a total of 27 sequences, which were clustered following the CD-HIT approach³⁹ to yield a pool of 21 representatives (from 15 source organisms, out of which 7 bacterial). Despite higher sequence divergence, multiple sequence alignment revealed that the GXXXGG motif is the most conserved feature also in this pool, suggesting that this is a universal signature of this particular type of AMPs (Fig. 2b; Supplementary Table 1c, d).

We can thus conclude that, based on sequence, A β peptides seem to share homology with a specific family of bacteriocins.

Mechanism of action. How could A β be beneficial against infected cells by its membrane penetrating ability? The majority of AMPs interact with the membrane directly without targeting specific receptors. Thanks to electrostatic and hydrophobic interactions, the peptide concentration on the bacterial membrane increases to reach a critical value that eventually promotes

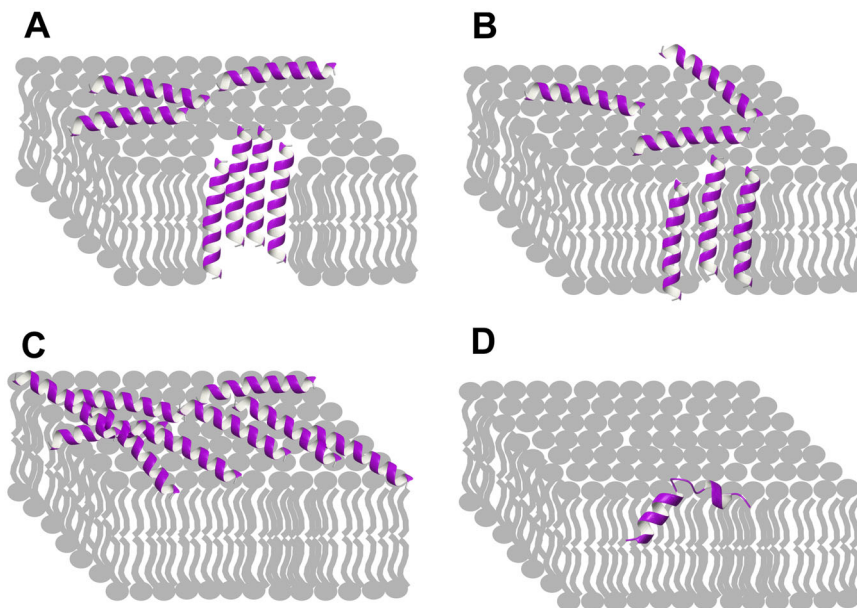


Fig. 3 Main mechanisms of action proposed for AMPs. **a** Barrel-stave model: helices of an AMP (violet) associate to form a pore; **b** toroidal model: helices interleave with lipids along the wall of the pore; **c** carpet model: helices cover the membrane surface as a carpet; **d** boomerang model: the kink joining two helices is instrumental for membrane penetration, only one of several boomerangs is shown.

membrane penetration according to pore or non-pore forming models. Some peptides that exhibit a random coil structure at neutral pH undergo a conformational transition at lower pH that results in an enhanced interaction with the membrane. This interaction promotes the formation of pores with consequent lysis of the membrane bilayers. The N-terminal peptide region of the hemagglutinin subunit of influenza, for instance, contains such a fusogenic sequence.

Pore models are often subdivided into the barrel-stave and toroidal models (Fig. 3a, b). In all of them the peptides are linear, without disulphide bridges and form one or more helices. The rare barrel-stave model, mainly represented by alamethicin, requires that amphipathic peptides assemble first on the surface of the membrane and successively insert perpendicularly through the lipid bilayer forming lateral peptide–peptide interactions. Toroidal models do not require specific peptide–peptide interactions, because the channel wall is formed both by peptide helices and lipids⁴⁰. Examples are melittin and magainin. Another popular mode of interaction with membranes, which does not require pore formation, is the so-called “carpet model”⁴¹, in which the peptides are adsorbed by the lipid bilayer until they cover the entire membrane, i.e., forming a “carpet” (Fig. 3c). Unfavourable interactions with the membrane surface eventually affect membrane integrity, leading to disruption accompanied by micelle formation.

Finally, a “boomerang model” was proposed for viral fusion domains (Fig. 3d) which undergo a conformational change at acidic pH leading to a V-shaped (boomerang) structure characterised by a kink⁶. Based on the structure of A β , we propose that this boomerang model could be crucial for its behaviour as an AMP.

Several viral peptides have been shown to directly ferry cargoes across the plasma membrane⁴². Examples include HIV-tat, the transcription factor Antennapedia, the VP22 protein from herpes simplex virus⁴³. Their peptidic regions responsible for cell penetration are either amphipathic (model amphipathic peptides or transportan) or arginine (Tat, VP22, penetratin) containing stretches of ~30 amino acids⁴². While the exact mechanism through which these peptides ferry either themselves or their conjugated payloads is unclear, it is indeed possible that their electrostatic interaction with the phospholipids might trigger the conformational change that allows insertion of these peptides into the membrane⁴⁴. Lipids of the host’s membrane or subcellular organellar membrane or the microbial membrane could be of paramount importance in the process. Several studies show that while some peptides could traverse the membrane through lipid interactions, conjugates of such peptides are mainly internalised via endocytosis^{45–47}. Akin to the influenza HA peptide or adenovirus capsid protein, A β could create havoc to the bacterial membrane and endosomal membranes of the infected cells. Several lipids, including the raft lipids GM1 and POPG, mediate the interaction both with the head groups and the tail⁴⁸. Once internalised, A β could, in its native form or a particular strain (GM1-A β , oligomeric A β), poke holes in the endosomal lipid bilayer rupturing the endosomal membrane^{49,50} through endo-osmolytic—wherein the cytoplasmic contents flow into the endosome causing rupture of the endosomal membrane. This would cause apoptosis or necroptosis of the infected cell, thus conferring protection against the infected cell and the microbe.

Conclusions

We have demonstrated here that both the sequence and the structure of the A β (1–42) peptide have strong similarities with AMPs from various organisms. A β peptides have been considered for a long time functionless byproducts of APP catabolism. This view mostly emerged because, when A β was first identified,

intramembrane cleavage was seen as an abnormal catabolic pathway. As a consequence, the production of A β was exclusively associated to a pathologic state. It is now accepted that intramembrane cleavage is a normal proteolytic pathway that generates different functionally important peptides. A β could thus be a normal constitutively generated peptide found in neurons. In support to this hypothesis is the evidence that the sequence of this region of APP is 100% conserved throughout most vertebrates⁵¹.

The assumption of A β (1–42) as an AMP provides a logical explanation for the presence of this peptide in our body and its concentration in neuronal synapses. It finally attributes an important and well-defined role to the peptide. In this scenario, A β peptides would be important components of the nervous system with several different functions, one of which being that as AMPs specialised in protecting the brain from foreigner attacks. Their release by the secretases would thus not be a mere accident that occurs only in concomitance with mutations. The experimental evidence in support of this thesis is getting increasingly convincing (there are for instance 28 reviews in Pubmed on “A β and antimicrobial peptide”, 16 of which published within the last 5 years).

Our findings are in turn compatible with several interesting not mutually exclusive scenarios. Based on the structural and sequence similarity with validated AMPs, we could for instance envisage that prolonged infection of bacteria (for instance from food but not only) that release A β -like bacteriocins could bring our immune system to develop anti-A β antibodies. Meanwhile neurons would develop A β as a physiologic important AMP to combat the bacterial attack. The similarity between A β and A β -like peptides would bring the immune response to turn its action against the neurons determining an autoimmune response and tissue damage. Alternatively, microbial A β -like peptides could favour A β oligomerization and provide a template for aberrant aggregation according to a prion-like mechanism. These and other hypotheses will need careful experimental validation which should lead to a better understanding of the AD aetiology.

In conclusion, the antimicrobial hypothesis¹⁰ proposes a completely different approach to AD from that so far adopted while not changing the nature of the molecular culprit: instead of eliminating A β or prevent its cleavage, an alternative strategy would be to identify possible preferential sources of infection. If this were possible, we could envisage to design vaccines to immunise people against these agents. Potential targets could for instance be proteins from *Porphyromonas gingivalis*, which has been suggested to be related to AD²⁰. A better understanding of the human microbiome might be essential to inform these studies.

Methods

Structures were retrieved from PDB (<https://www.rcsb.org/>) and visualised by the MolMol software (<https://sourceforge.net/projects/molmol/>). Sequence similarity searches were carried out using PSIBLAST (<https://www.ebi.ac.uk/Tools/sss/psiblast/>). Multiple sequence alignments were generated by Expresso/TCoffee (<http://tcoffee.crg.cat/apps/tcoffee/do:expresso>) and visualised through Jalview (<http://www.jalview.org/getdown/release/>).

Data availability

The data sets analysed in the current study are available in the dbAMP repository (<https://doi.org/10.1093/nar/gky1030>). These data sets were derived from the following public domain resources: <http://140.138.77.240/~dbamp/index.php>.

Code availability

All software used in this paper is freely available on the web.

Received: 11 November 2019; Accepted: 2 March 2020;

Published online: 19 March 2020

References

- Hardy, J. & Selkoe, D. J. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* **297**, 353–356 (2002).
- Spinney, L. Alzheimer's disease: the forgetting gene. *Nature* **510**, 26–28 (2014).
- Doig, A. J. & Derreumaux, P. Inhibition of protein aggregation and amyloid formation by small molecules. *Curr. Opin. Struct. Biol.* **30**, 50–56 (2015).
- Morgado, I. & Garvey, M. Lipids in amyloid- β processing, aggregation, and toxicity. *Adv. Exp. Med. Biol.* **855**, 67–94 (2015).
- Crescenzi, O. et al. The Alzheimer amyloid beta-peptide as a virus fusion domain: solution structure of amyloid beta-peptide(1–42) in an apolar microenvironment. *Eur. J. Biochem.* **269**, 5642–5648 (2002).
- Han, X. et al. Membrane structure and fusion-triggering conformational change of the fusion domain from influenza hemagglutinin. *Nat. Struct. Biol.* **8**, 715–720 (2001).
- Temussi, P. A. et al. From Alzheimer to Huntington: why is a structural understanding so difficult? *EMBO J.* **22**, 355–61. (2003).
- Li, Y. Membrane structures of the hemifusion-inducing fusion peptide mutant G1S and the fusion-blocking mutant G1V of influenza virus hemagglutinin suggest a mechanism for pore opening in membrane fusion. *J. Virol.* **79**, 12065–76. (2005).
- Kumar, D. K. et al. Amyloid- β peptide protects against microbial infection in mouse and worm models of Alzheimer's disease. *Sci. Transl. Med.* **8**, 340ra72 (2016).
- Moir, R. D. et al. The antimicrobial protection hypothesis of Alzheimer's disease. *Alzheimers Dement.* **14**, 1602–1614 (2018).
- Jamieson, G. A., Maitland, N. J., Wilcock, G. K., Craske, J. & Itzhaki, R. F. Latent herpes simplex virus type 1 in normal and Alzheimer's disease brains. *J. Med. Virol.* **33**, 224–227 (1991).
- Soscia, S. J. et al. The Alzheimer's disease associated amyloid beta-protein is an antimicrobial peptide. *PLoS One* **5**, e9505 (2010).
- Bourgade, K. et al. Protective effect of amyloid- β peptides against herpes simplex virus-1 infection in a neuronal cell culture model. *J. Alzheimers Dis.* **50**, 1227–41. (2016).
- Bourgade, K. et al. β -Amyloid peptides display protective activity against the human Alzheimer's disease-associated herpes simplex virus-1. *Biogerontology* **16**, 85–98 (2015).
- White, M. R. et al. Alzheimer's associated β -amyloid protein inhibits influenza A virus and modulates viral interactions with phagocytes. *PLoS One* **9**, e101364 (2014).
- Fulop, T. et al. Can an infection hypothesis explain the beta amyloid hypothesis of Alzheimer's disease? *Front. Aging Neurosci.* **10**, 224 (2018).
- Capsoni, S., Carucci, N. M. & Cattaneo, A. Pathogen free conditions slow the onset of neurodegeneration in a mouse model of nerve growth factor deprivation. *J. Alzheimers Dis.* **31**, 1–6 (2012).
- Emery, D. C. et al. 16S rRNA next generation sequencing analysis shows bacteria in Alzheimer's post-mortem brain. *Front. Aging Neurosci.* **9**, 195 (2017).
- Osorio, C. et al. The post-amyloid era in Alzheimer's disease: trust your gut feeling. *Front. Aging Neurosci.* **11**, 143 (2019).
- Dominy, S. S. et al. Porphyromonas gingivalis in Alzheimer's disease brains: evidence for disease causation and treatment with small-molecule inhibitors. *Sci. Adv.* **5**, eaau3333 (2019).
- Zeya, H. I. & Spitznagel, J. K. Antibacterial and enzymic basic proteins from leukocyte lysosomes: separation and identification. *Science* **142**, 1085–1087 (1963).
- Zeya, H. I. & Spitznagel, J. K. Arginine-rich proteins of polymorphonuclear leukocyte lysosomes: antimicrobial specificity and biochemical heterogeneity. *J. Exp. Med.* **127**, 927–941 (1968).
- Wang, G. Human antimicrobial peptides and proteins. *Pharmaceuticals* **7**, 545–594 (2014).
- Coles, M. et al. Solution structure of amyloid b-peptide (1–40) in a water micelle environment. Is the membrane-spanning domain where we think it is? *Biochemistry* **37**, 11064–11077 (1998).
- Shao, H. et al. Solution structures of micelle-bound amyloid b-(1–40) and b-(1–42) peptides of Alzheimer's disease. *J. Mol. Biol.* **285**, 755–773 (1999).
- Sticht, H. et al. Structure of amyloid A4-(1–40) -peptide of Alzheimer's disease. *Eur. J. Biochem.* **233**, 293–298 (1995).
- Epand, R. M. & Vogel, H. J. Diversity of antimicrobial peptides and their mechanisms of action. *Biochim. Biophys. Acta* **1462**, 11–28 (1999).
- Steinhauer, D. A. et al. Studies of the membrane fusion activities of fusion peptide mutants of influenza virus hemagglutinin. *J. Virol.* **69**, 6643–6651 (1995).
- Terwilliger, T. C. & Eisenberg, D. The structure of melittin. II. Interpretation of the structure. *J. Biol. Chem.* **257**, 6016–6022 (1982).
- Bazzo, R. et al. The structure of melittin. A 1H-NMR study in methanol. *Eur. J. Biochem.* **173**, 139–46. (1988).
- Brown, L. R. et al. High-resolution 1H-NMR studies of self-aggregation of melittin in aqueous solution. *Biochim. Biophys. Acta* **622**, 231–44 (1980).
- Kim, J. K. et al. Structure and function of papiliocin with antimicrobial and anti-inflammatory activities isolated from the swallowtail butterfly, Papilio xuthus. *J. Biol. Chem.* **286**, 41296–41311 (2011).
- Terry, A. S. et al. The cDNA sequence coding for prepro-PGS (prepro-magainins) and aspects of the processing of this prepro-polypeptide. *J. Biol. Chem.* **263**, 5745–5751 (1988).
- Williams, R. W. et al. Raman spectroscopy of synthetic antimicrobial frog peptides magainin 2a and PGLa. *Biochemistry* **29**, 4490–4496 (1990).
- Gesell, J. et al. Two-dimensional 1H NMR experiments show that the 23-residue magainin antibiotic peptide is an alpha-helix in dodecylphosphocholine micelles, sodium dodecylsulfate micelles, and trifluoroethanol/water solution. *J. Biomol. Nmr.* **9**, 127–135 (1997).
- Wang, G. Structures of human host defense cathelicidin LL-37 and its smallest antimicrobial peptide KR-12 in lipid micelles. *J. Biol. Chem.* **283**, 32637–32643 (2008).
- Bhattacharjya, S., Venkatraman, J., Kumar, A. & Balaran, P. Fluoroalcohols as structure modifiers in peptides and proteins: hexafluoroacetone hydrate stabilizes a helical conformation of melittin at low pH. *J. Pept. Res.* **54**, 100–111 (1999).
- Altschul, S. F. et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25**, 3389–3402 (1997).
- Li, W. & Godzik, A. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics* **22**, 1658–1659 (2006).
- Kumar, P. et al. Antimicrobial peptides: diversity, mechanism of action and strategies to improve the activity and biocompatibility in vivo. *Biomolecules* **8**, pii: E4 (2018).
- Shai, Y. Mode of action of membrane active antimicrobial peptides. *Biopolymers* **66**, 236–248 (2002).
- Gupta, B., Levchenko, T. S. & Torchilin, V. P. Intracellular delivery of large molecules and small particles by cell-penetrating proteins and peptides. *Adv. Drug Deliv. Rev.* **57**, 637–651 (2005).
- Elliott, G. & O'Hare, P. Intercellular trafficking and protein delivery by a herpesvirus structural protein. *Cell* **88**, 223–233 (1997).
- Wender, P. A. et al. The design, synthesis, and evaluation of molecules that enable or enhance cellular uptake: peptoid molecular transporters. *Proc. Natl Acad. Sci. USA* **97**, 13003–13008 (2000).
- Chen, C. & Zhuang, X. Epsin 1 is a cargo-specific adaptor for the clathrin-mediated endocytosis of the influenza virus. *Proc. Natl Acad. Sci. USA* **105**, 11790–11795 (2008).
- Duchardt, F., Fotin-Mlecsek, M., Schwarz, H., Fischer, R. & Brock, R. A comprehensive model for the cellular uptake of cationic cell-penetrating peptides. *Traffic* **8**, 848–866 (2007).
- Holowka, E. P., Sun, V. Z., Kamei, D. T. & Deming, T. J. Polyarginine segments in block copolypeptides drive both vesicular assembly and intracellular delivery. *Nat. Mater.* **6**, 52–57 (2007).
- Kagan, B. L., Hirakura, Y., Azimov, R., Azimov, R. & Lin, M. C. The channel hypothesis of Alzheimer's disease: current status. *Peptides* **23**, 1311–1315 (2002).
- Mastrobattista, E. et al. Functional characterization of an endosome-disruptive peptide and its application in cytosolic delivery of immunoliposome-entrapped proteins. *J. Biol. Chem.* **277**, 27135–27143 (2002).
- Leopold, P. L. & Crystal, R. G. Intracellular trafficking of adenovirus: many means to many ends. *Adv. Drug Deliv. Rev.* **59**, 810–821 (2007).
- Luna, S. et al. Amyloid- β and APP deficiencies cause severe cerebrovascular defects: important work for an old villain. *PLoS One* **8**, e75052 (2013).
- Koradi, R. et al. MOLMOL: a program for display and analysis of macromolecular structure. *J. Mol. Graph.*, **14**, 51–55 (1996).
- Notredame, C. et al. T-Coffee: a novel method for fast and accurate multiple sequence alignment. *J. Mol. Biol.* **302**, 205–217 (2000).
- Waterhouse, A. M. et al. Jalview version 2—a multiple sequence alignment editor and analysis workbench. *Bioinformatics* **25**, 1189–1191 (2009).

Acknowledgements

This work was supported by Dementia Research UK (RE1 3556). F.R. would like to thank Dr. Ranka Vukotic and Prof. Ferruccio Bonino for inspiring discussions. LR acknowledges the UKDRI and the van Geest Foundation for grants.

Author contributions

A.P. suggested the topic, F.R. carried out the sequence analysis, L.R. took care of the mechanism of A β , P.A.T. wrote the first version of the paper. All authors contributed to finalise the text.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at <https://doi.org/10.1038/s42003-020-0865-9>.

Correspondence and requests for materials should be addressed to A.P. or P.A.T.

Reprints and permission information is available at <http://www.nature.com/reprints>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2020