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Smart Diaspora 2023

10 - 13 Aprilie 2023, Timișoara

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Universitatea de Vest din Timișoara

Universitatea de Medicină și Farmacie Victor Babeș | Timișoara

Universitatea Politehnica Timișoara

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CRP-a key protagonist of neurovascular dysfunction and AD pathogenesis

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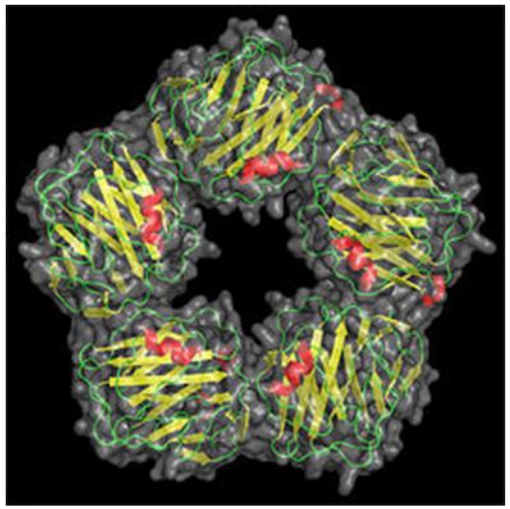
Mark Slevin FRCPath, PhD

University of Medicine, Pharmacy, Science and Technology of Târgu Mureș

CRP-a key protagonist of neurovascular dysfunction and AD pathogenesis

- C-reactive protein (CRP) is an acute phase pentameric protein produced by the liver in response to inflammation[1]



- 5 identical monomers held together by disulphide bonds
  - Total mass 120,000 Da

1. Thiele R T. *et al.* «Dissociation of Pentameric to Monomeric C-Reactive Protein Localizes and Aggravates Inflammation - In Vivo Proof of a Powerful Proinflammatory Mechanism and a New Anti-Inflammatory Strategy.» *Circulation* (2014): 130, N° 1.

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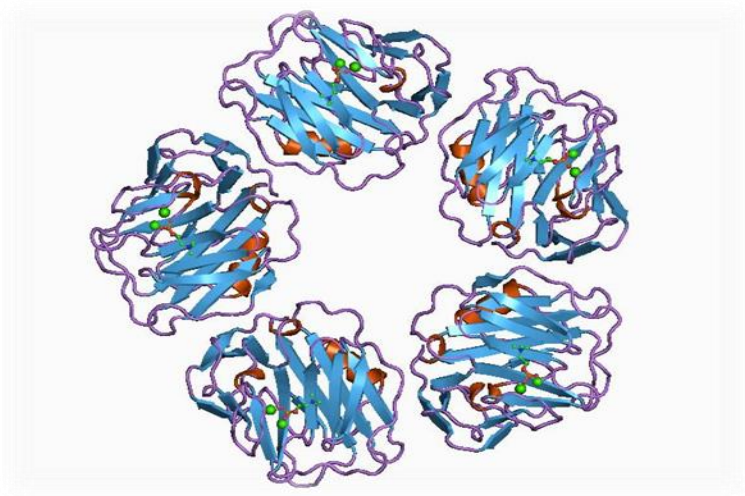
- Native CRP (nCRP) dissociates irreversibly to its monomeric subunits (mCRP) which display more highly active biological properties

- Dissociation occurs on contact with activated tissues or cells at sites of inflammation and infection[1]



- Breaking of the disulfate bonds results in monomers which bind during the activation process to phosphatidylcholine on the surface of cells

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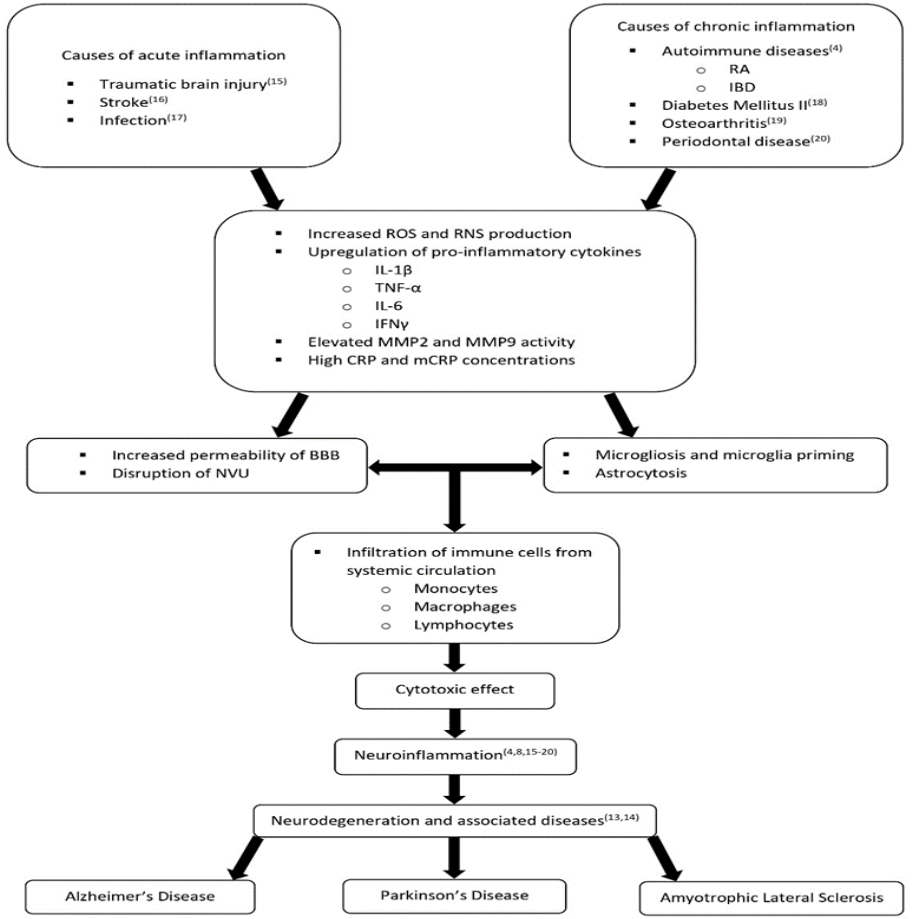
Inflammation and vascular dementia:



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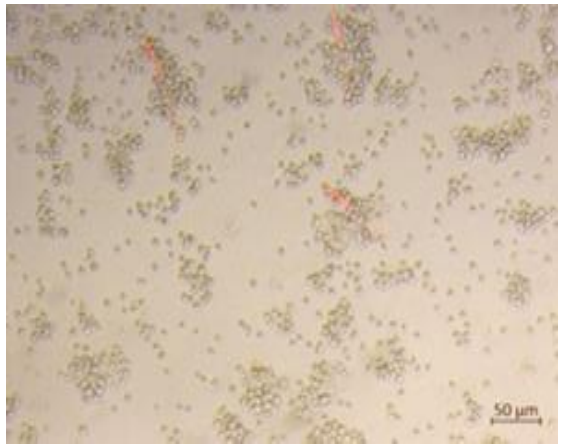
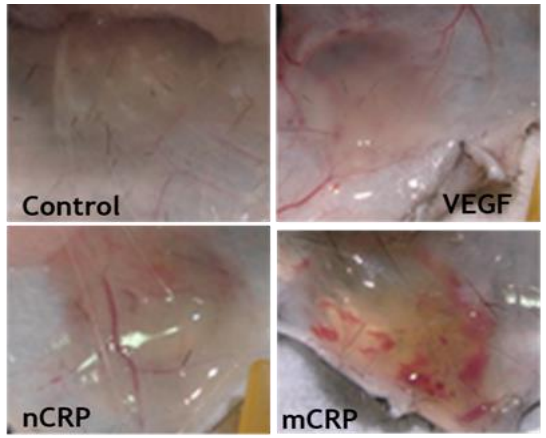
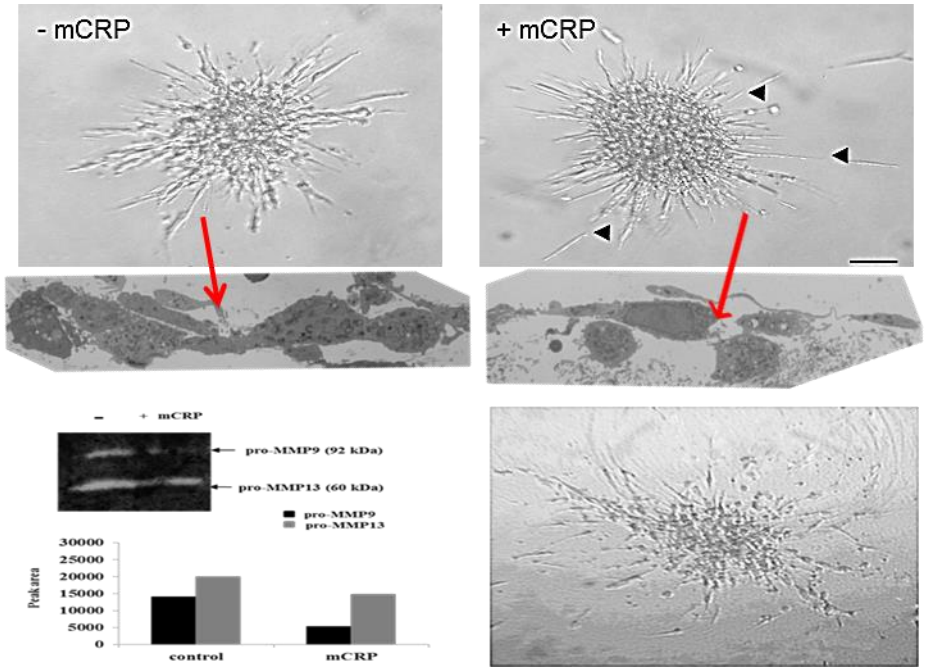
mCRP is HIGHLY biologically active!



Monocyte (above), and platelet aggregation.



Causes vascular heamorrhage *in vivo*.



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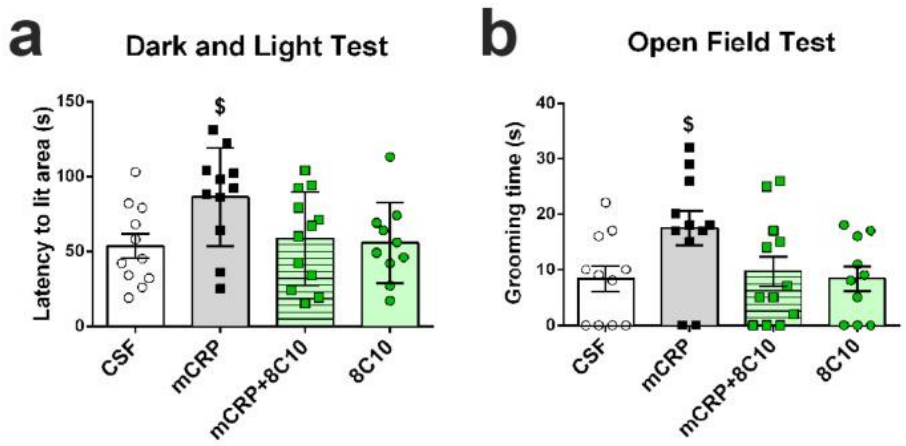
mCRP in vascular dementia:



Dark and light test- normal exploratory behavior of wild-type mice

Open field test- grooming time and locomotor activity

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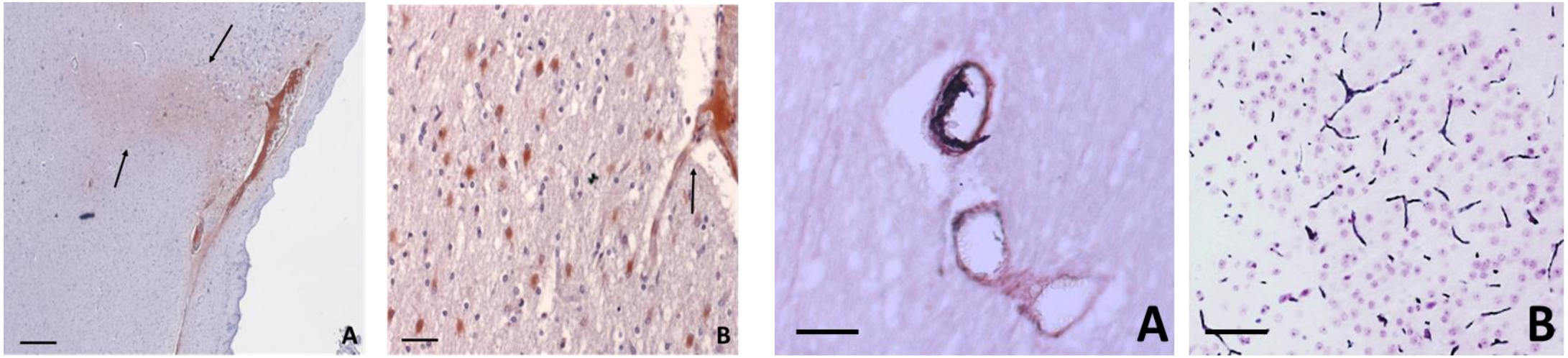

Microvascular ‘pockets’ of mCRP - infiltrating the local cerebral parenchyma (arrows) - magnified appearance of a leaking cortical microvessel with localised mCRP-positive inflammatory reaction and immune-infiltration- Luminal mCRP in infarcted brain and – cortical microvessels, positively stained for mCRP

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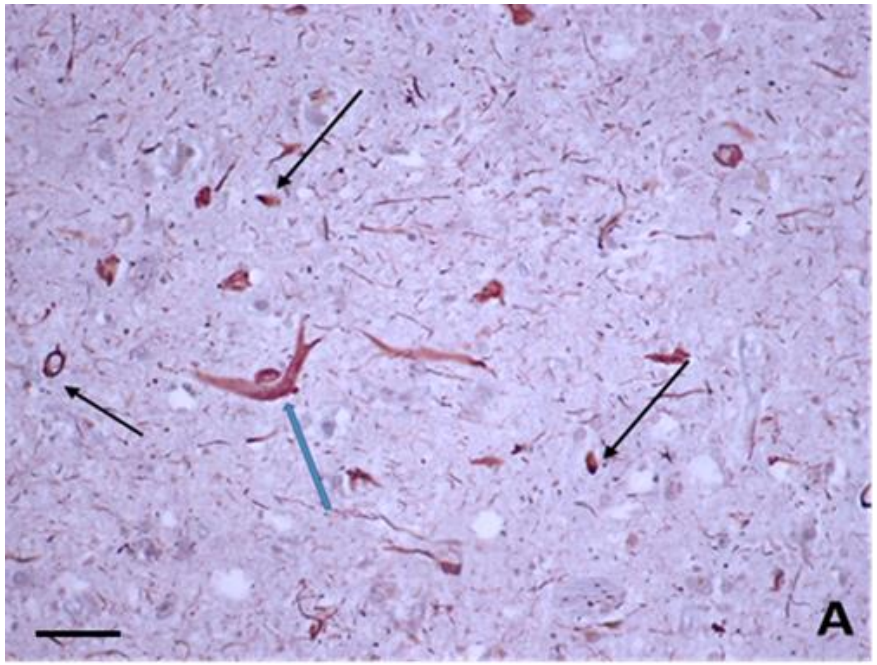

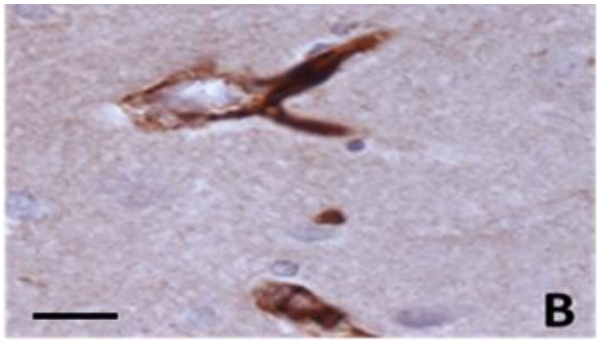

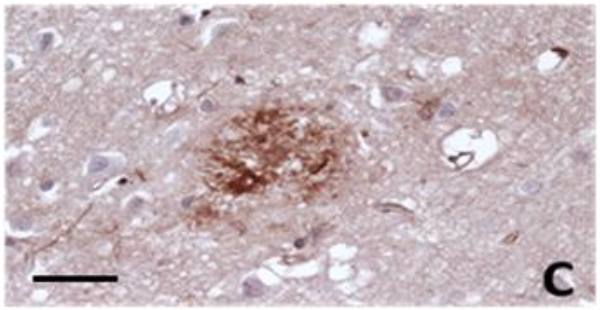

Chronic extra-cellular expression of mCRP in the cortical tissue stroked AD brain, from 12. (A) showing strong staining in neuritic plaques, fibril-like structures and immune infiltrating cells

(B), is a magnified image of a neuritic plaque intensely ‘coated’ with mCRP and (C), mCRP positive region with the appearance of a cortical plaque.

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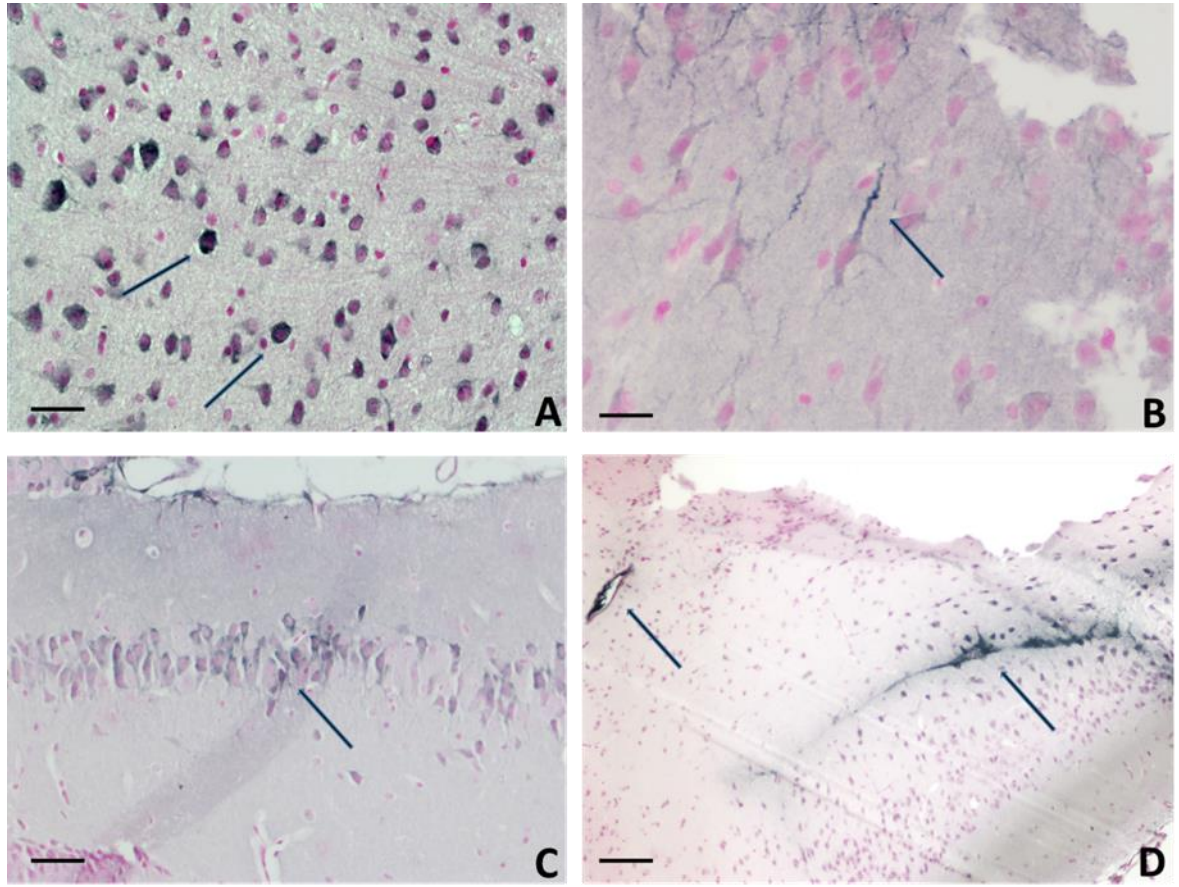

mCRP was stereotactically delivered into the hippocampal CA1 region of wild-type mice and morphological and topological localization was observed by IHC (DAB grey-black development) after 1-6 months.

Nuclear expression is seen in neocortical neurons (A) with positive axonal staining (A-B) and in (C), mCRP-peri-nuclear staining of hippocampal neurons in CA1 and (D), shows mCRP near the injection site (arrow on the right) and strong expression in an adjacent blood vessel (left arrow) and dorsal and lateral ventricles

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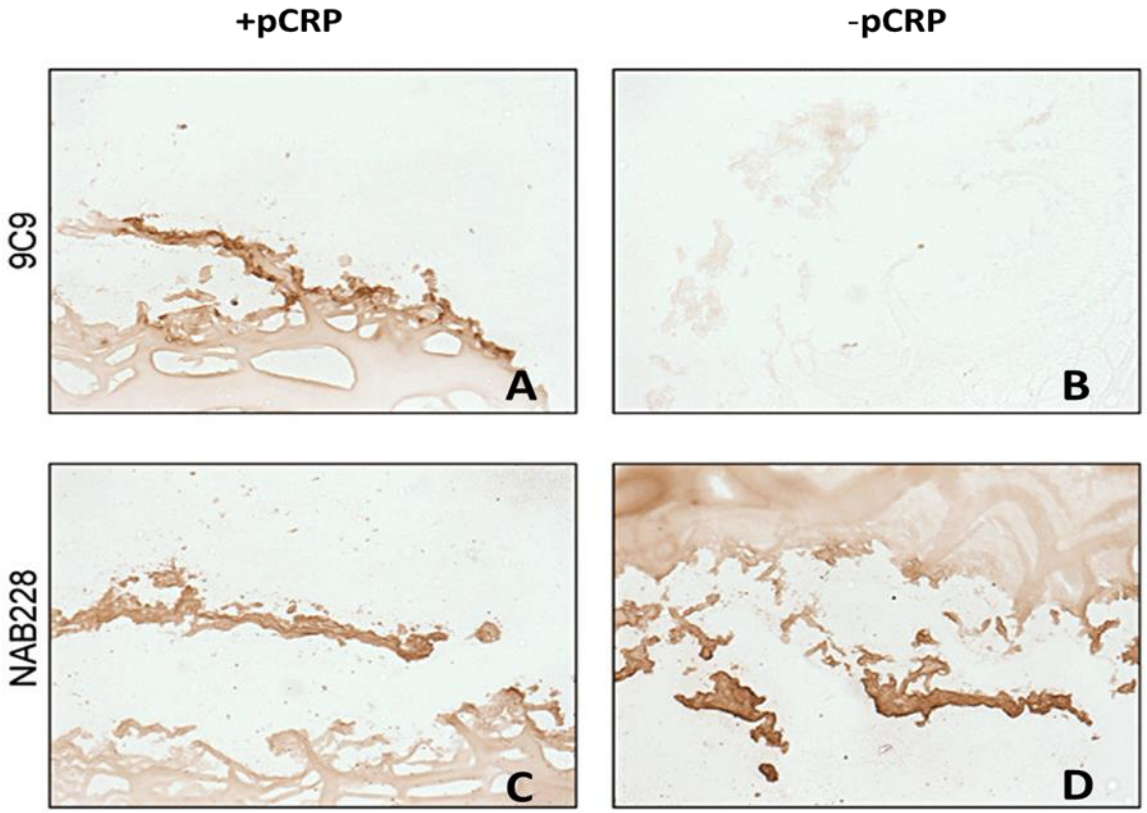

An artificial Aβ plaque incubated with pCRP- (A) and fixed in agarose showing positive (DAB-brown) staining with antibody clones 9C9 (mCRP) and NAB228 (Aβ)

However, plaques incubated without pCRP- (B-D), showed no staining for 8D8 or 9C9 (B).

Strang et al, 2012

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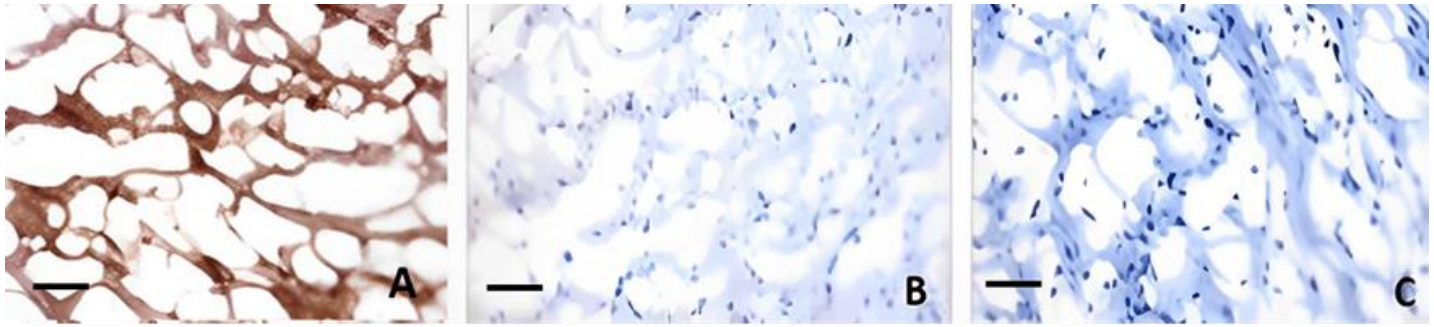
mCRP modulation in dementia:



mCRP following Myocardial infarction in rats

When pCRP was preincubated with 1,6-bis-PC (50:1 molar ratio), there was no significant deposition of mCRP or pCRP in either the infarcted (C) or non-infarcted tissue.

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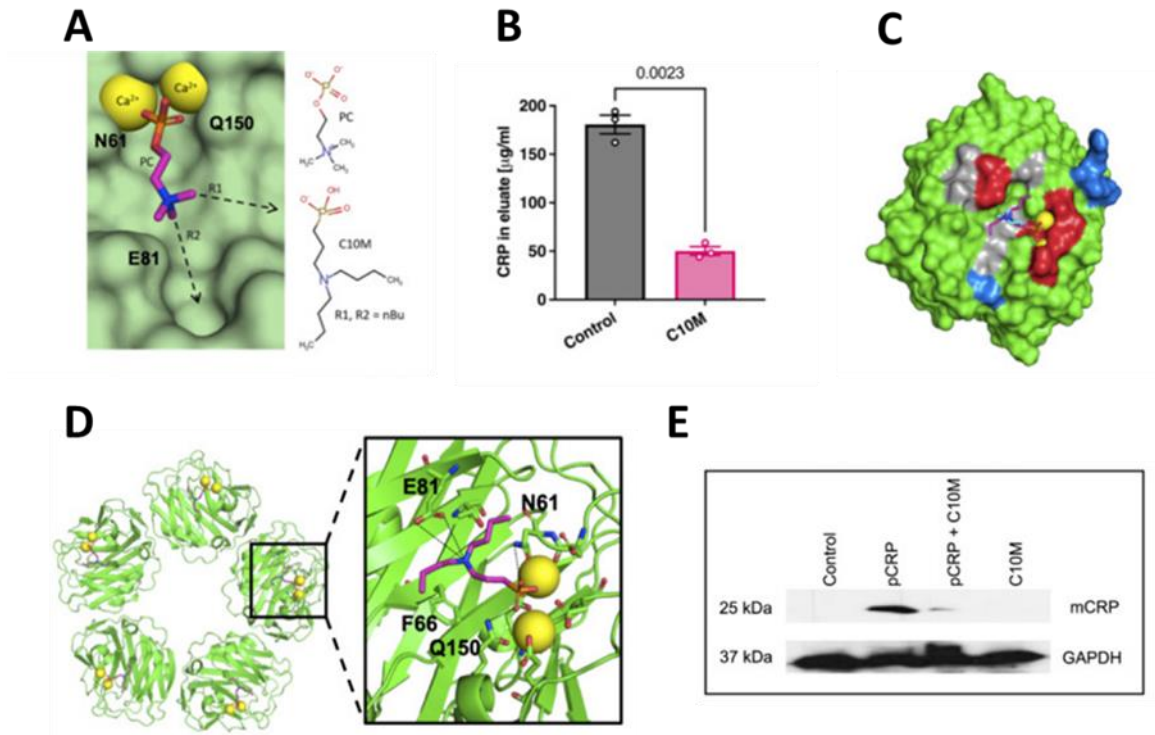

Design of the phosphonate compound C10M

Based on binding characteristics of PC:pCRP and two n-butyl substituents on the tertiary amine in the binding pocket via vectors R1 and R2.

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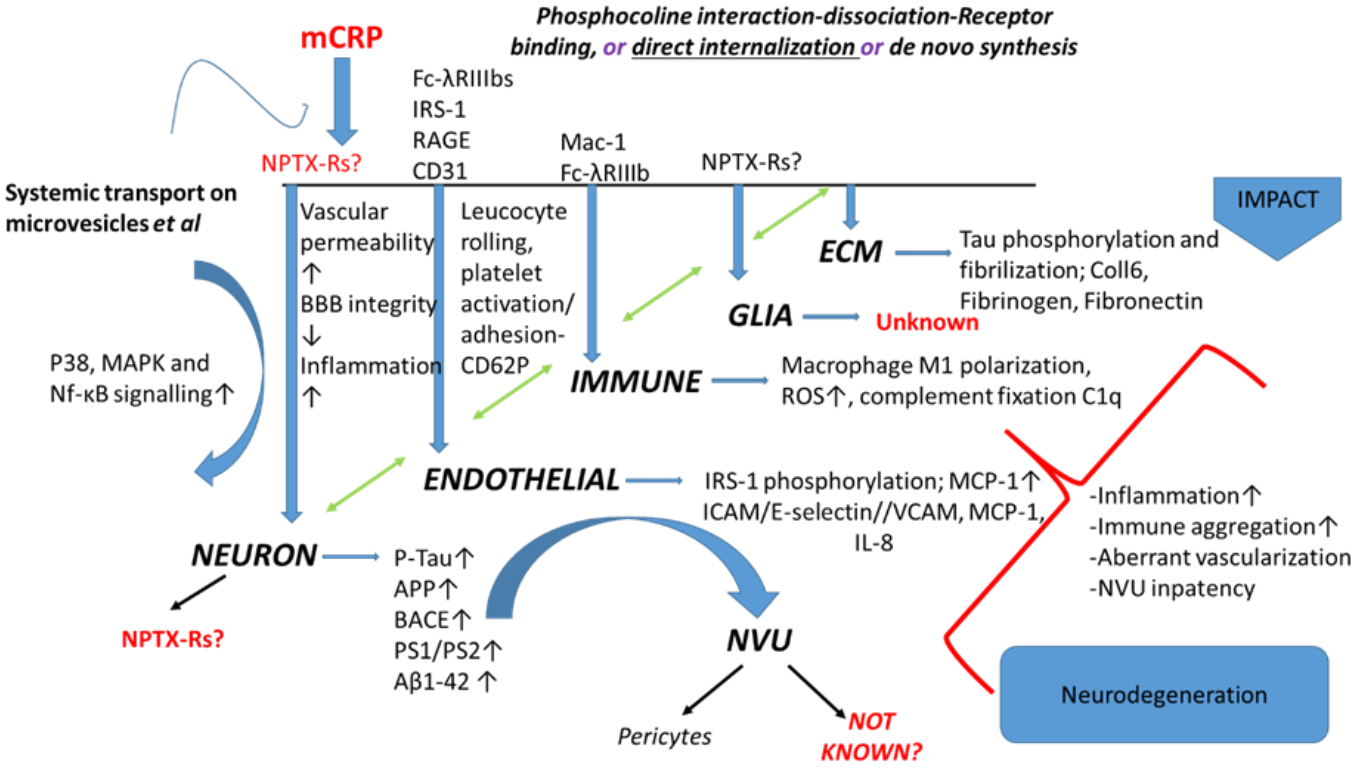

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mCRP in vascular dementia:

SMIs could reach constant therapeutically active levels beyond the BBB, protect the brain vasculature and enhance NVU stability in acute inflammatory pathologies such as stroke, and/ or chronic inflammation as in auto-inflammatory disease remains unknown;

Limitation might be that mCRP could start to be ‘laid down’ in brain tissue early in the disease process and would seem to remain stably (as morphological and histological studies have shown) with no known mechanism to remove.

In any case, protection of vulnerable individuals by risk stratification or clinical history and reduction of subsequent risk of acute vascular perturbation might be possible as we learn more about the mechanisms of cellular activation along with conduction of proof-of-concept studies.

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OUR ONGOING WORK

1. **Characterise the impact of mCRP and associated neuro-vascular-inflammation on NVU function *in vitro***, specifically, through analysis of pericyte-vascular interaction and morphology associated with vessel contraction, patency (leakage/haemorrhage- BBB etc.), and other dysfunction, -these could impact upon intramural peri-arterial drainage capacity (IPAD).

2. First stage-proof-of-concept *in vivo*, utilizing murine models of neurodegeneration, mCRP-wild-type, ApoE4 knock in model +/- mCRP [for NVU disruption with a stage by stage analysis], and triple mutant transgenic (3 x Tg-AD; PS1M146V, APPSwe, and tauP301L) standard AD models, - **to ascertain the impact of mCRP within the development of neurovascular pathology**.

3. Characterization of AD post-mortem samples in subjects who died with AD; using a cohort of cortical tissue slices we will confirm the above findings from 1) and 2), and use IHC to **detail the critical component proteins, their expression, co-localization and their association with vascular and NVU dysfunction.**

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