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## Porcine SAA is not likely an apolipoprotein bound to circulating HDL3

Laura Soler, Ana Gutiérrez, P. David Eckersall, Nicola Merola, José J. Cerón,  
Théo Niewold

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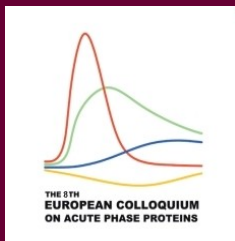
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# “Porcine Serum Amyloid A is not likely an apolipoprotein bound to circulating HDL”

**Laura Soler, Ana Gutiérrez, P. David Eckersall, Nicola Merola, Jose J Cerón, Theo Niewold**



8th European Colloquium on Acute Phase Proteins  
Helsinki, August 26th 2010

# *Veterinary clinical analysis research group, University of Murcia*



*Theo Niewold*

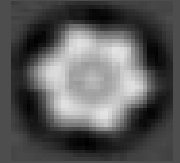
*David Eckersall*

*Nicola Merola*

*University of Leuven University of Glasgow University of Murcia*



# SERUM AMYLOID A (SAA)



Acute phase, 14 kDa protein, multimers

## SAA1 and SAA2

Mainly produced in liver  
Circulating  
Apolipoproteins  
Neutral IEP

## SAA3

Mainly produced locally  
Not circulating  
Alkaline IEP

Different functions



# PORCINE SAA: THE BIG UNKNOWN



- Purification of pig SAA not published
- Nucleotide and amino acid sequences of each SAA isoform has not been reported
- No reports on identification by proteomic techniques

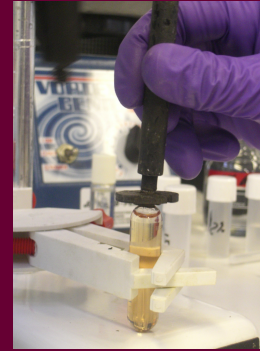
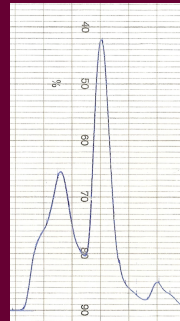
Mass spectral analysis of pig (*Sus scrofa*) apo HDL: Identification of pig apoA-II, a dimeric apolipoprotein

Donald L. Puppione<sup>a,\*</sup>, Julian P. Whitelegge<sup>b</sup>, Lang M. Yam<sup>a</sup>, Verne N. Schumaker<sup>a</sup>

- *Importance*: swine resistance to amyloidosis development

# OBJECTIVES

- 1 Purifying SAA from porcine serum using traditional protocols and study its properties



- 2 Analyzing predicted properties from sequences deposited in UniProtKB database by using ExPASy server sequence analysis tools



## SAA purification from pig serum

### 1- Guanidine hydrochloride-eluted HIC

The acute phase serum amyloid A protein (SAA) in the horse: isolation and characterization of three isoforms

C. Hultén <sup>a,\*</sup>, K. Sletten <sup>b</sup>, C. Foyen Bruun <sup>c</sup>, G. Marhaug <sup>c</sup>

### 2- Ethanol-eluted HIC

Use of Ethanol-Eluted Hydrophobic Interaction Chromatography in the Purification of Serum Amyloid A

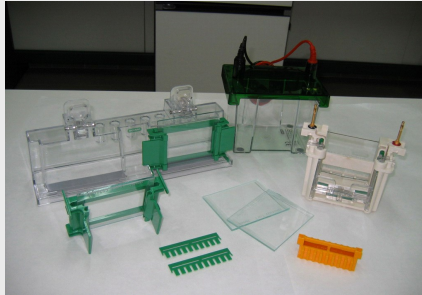
James W. Smith and Thomas L. McDonald

### 3- Gradient density ultracentrifugation using

Separation of Bovine Plasma Lipoproteins by a Rapid Ultracentrifugation Method

R. S. Gardner, N. H. Ogden\*, P. J. Cripps and D. Billington†

# SDS-PAGE and immunoblotting analysis



1. Rabbit anti-bovine SAA3 pAb Dr. Molenaar

agresearch Farming, Food and Health. First™

2. Rat anti-porcine recombinant SAA mAb

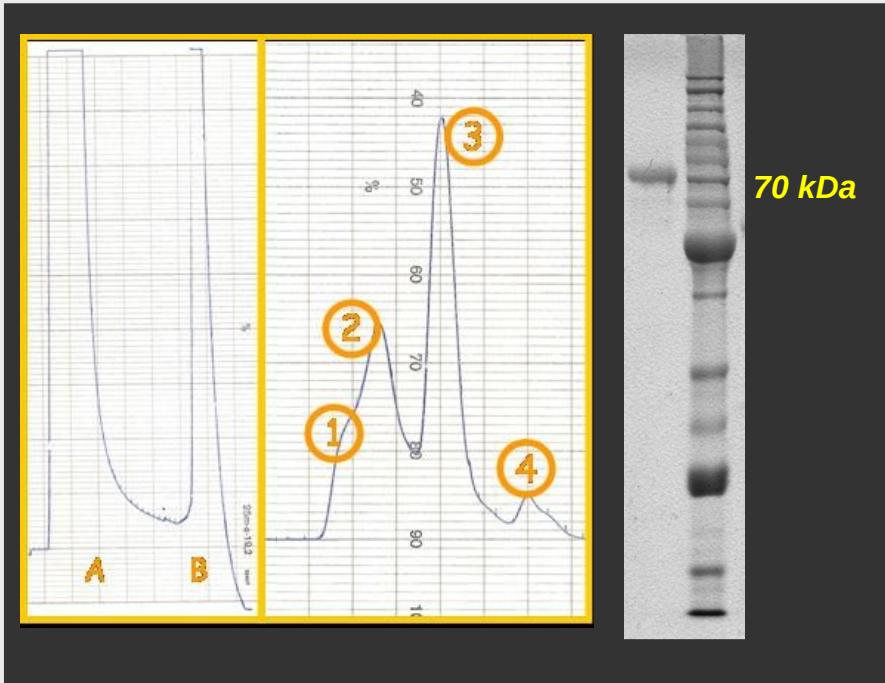
## Sequence analysis

Search UniProtKB database for porcine SAA sequences and analyze with ExPASy tools (ProtParam, GRAVY)

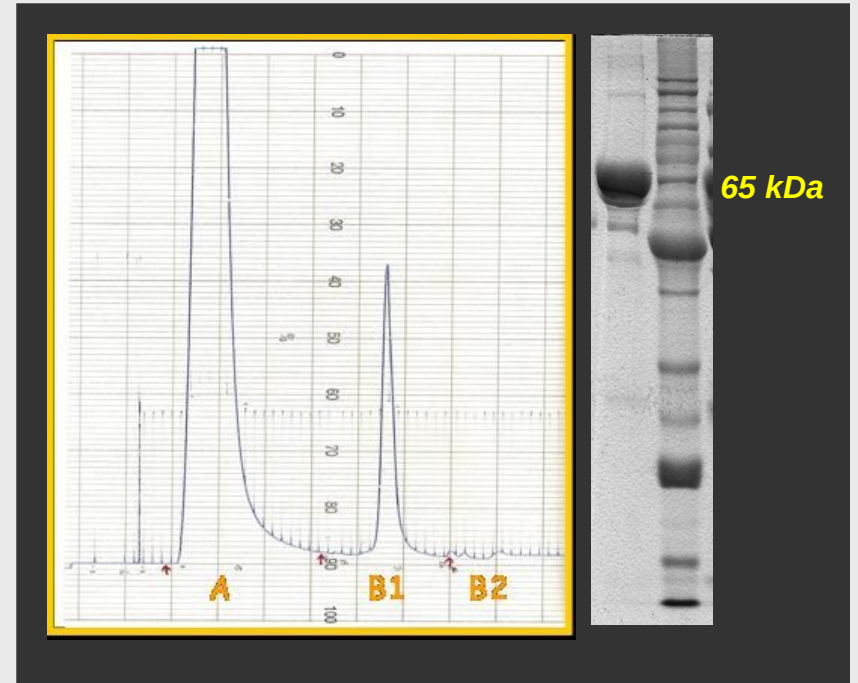


## Results

- Hydrophobic interaction chromatography



Guanidine hydrochloride

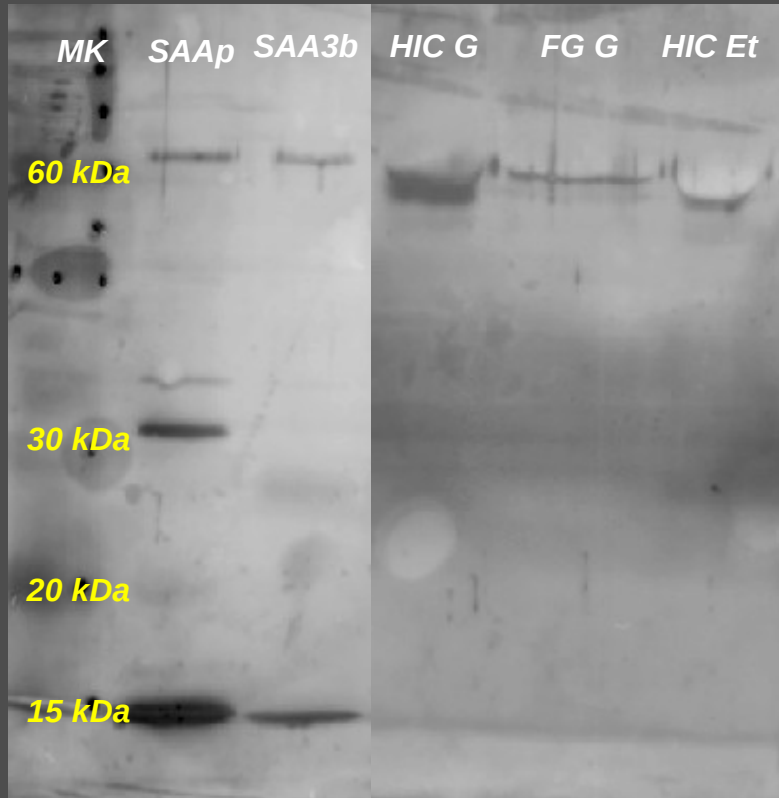
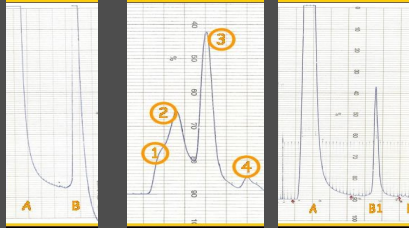


Ethanol

## Results

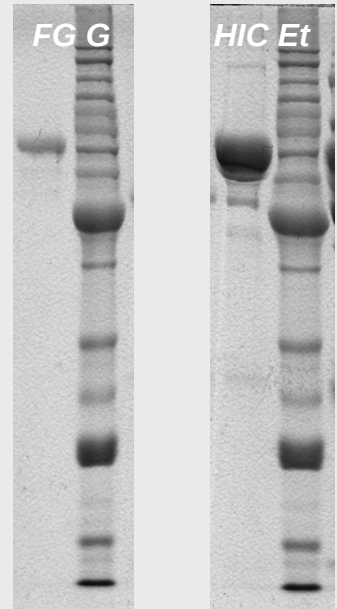
### • Immunoblotting analysis

#### Rabbit pAb



#### Rat mAb

MK  
SAA3b  
HIC G  
FG G  
HIC Et



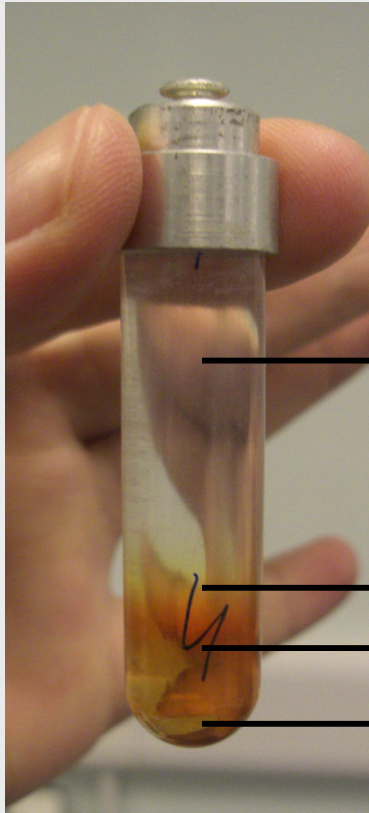
SDS-PAGE

## Results from HIC chromatography:

- Porcine SAA binds to the HIC columns and need chaotropic agents, like guanidine hydrochloride, to elute.
- The purification showed has a very low throughput and yield
- It seems that the bulk of the SAA remain unbound to the HIC columns and is lost during the process

Results

• Gradient Density Ultracentrifugation

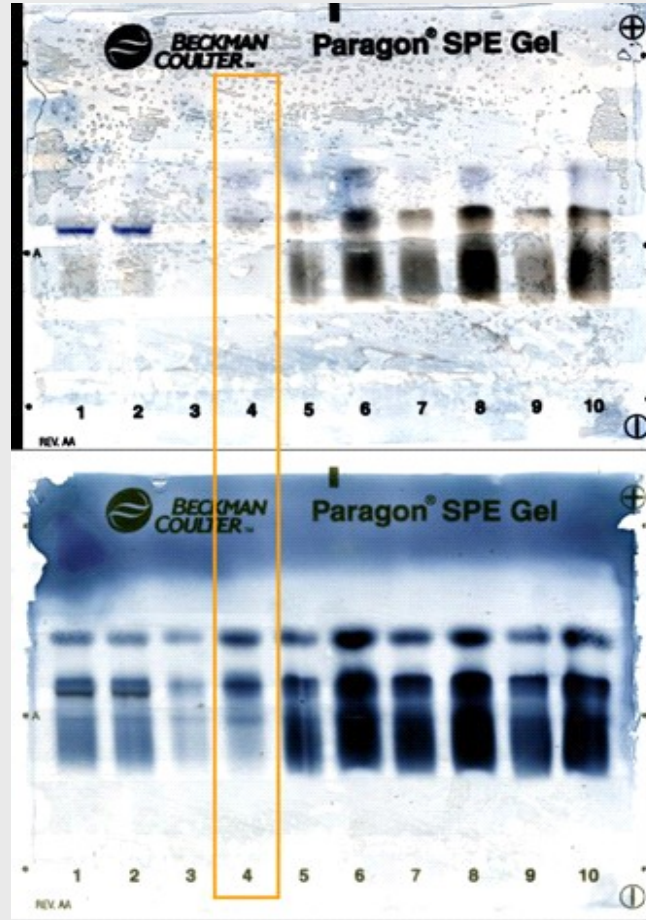


→ VLDL

→ LDL

→ **HDL**

→ Proteins



Sudan

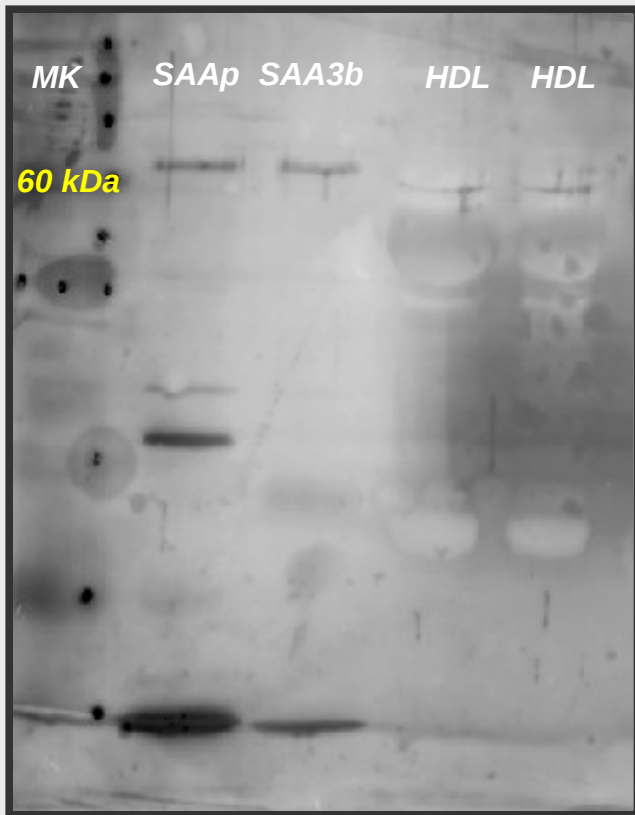
Coomassie

Agarose gel electrophoresis

## Results

- Immunoblotting analysis

**Rabbit pAb**

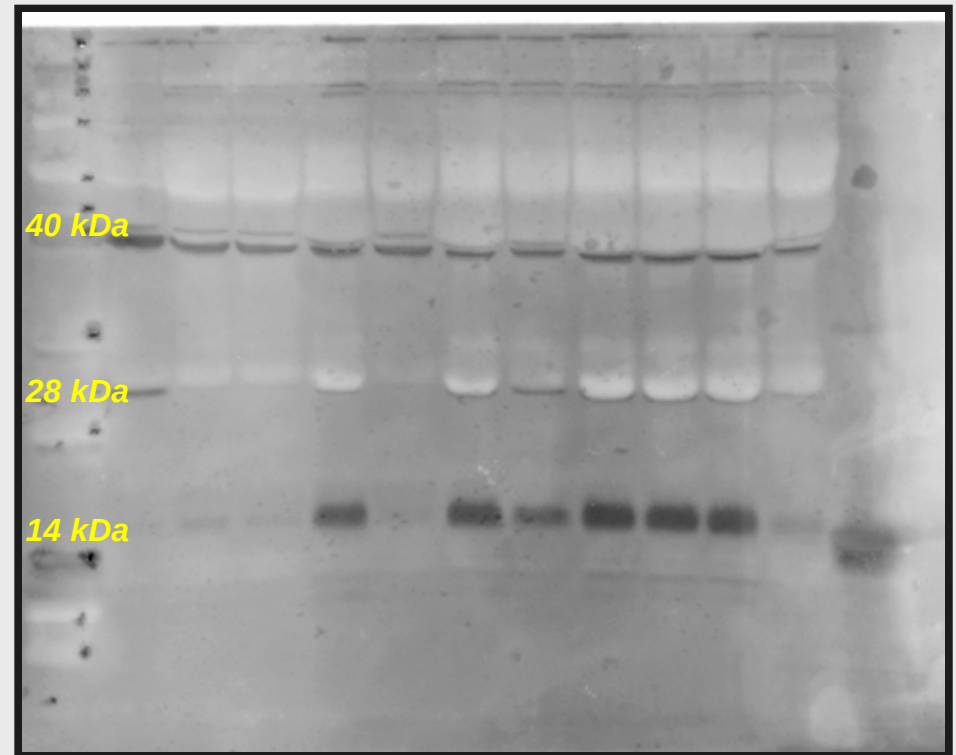


**Rat mAb**

*HDL*



*Proteins*



## Results from SAA-HDL isolation by gradient density ultracentrifugation:

- Porcine SAA is present in HDL-rich fractions

Mass spectral analysis of pig (*Sus scrofa*) apo HDL: Identification of pig apoA-II, a dimeric apolipoprotein

Donald L. Puppione<sup>a,\*</sup>, Julian P. Whitelegge<sup>b</sup>, Lang M. Yam<sup>a</sup>, Verne N. Schumaker<sup>a</sup>

- Immunoblot analysis indicates that the bulk of SAA in porcine serum is not bound to HDL, but soluble and present in the serum protein layer

## Results

### • Sequence analysis

① Q2HXZ9 Chang et al., 2007, **Liver**, 130 aa, translated from

② B9P414 Rodriguez et al., 2007, **Mammary gland**, 89 aa, translated from mRNA

B9P414

Q2HXZ9

```
1 -----SDMREANYKNS 11
                                     |||
1 MKLSTGIIFCFLILGVSSQRWASFLKEAGQGAKDMWRAYSDMREANYKNS 50
12 DKYFHARGNYDAARRGPGGAWAAKVISDARENVQRVTDLFKHGDSGHGVE 61
   |||:|||||
51 DKYFHARGNYDAARQRGPGGAWAAKVISDARENVQRVTDLFKHGDSGHGVE 100

62 DSRADQAANAWGRSGKDPNHFRPRGLPD-- 89
   |||
101 DSRADQAANAWGRSGKDPNHFRPRGLPDKY 130
```

MKLSTGIIFCFLILGVSSQRWASFLKEAGQGAKDMWRAYS  
SDMREANYKNSDKYFHARGNYDAAQRGPGGAWAAKVISDARENVQRVTDLFKHGDSGHGVEDSRADQAANAWGRSGKDPNHFRPRGLPDKY

## Results

- Sequence analysis

Q2HXZ9

**Molecular weight: 14511.1**

**Theoretical pI: 9.48**

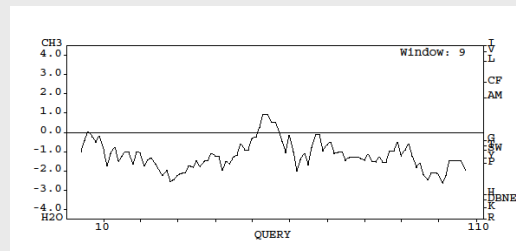
**Grand average of hydropathicity (GRAVY): -0.86**

B9P414

**Molecular weight: 9854.6**

**Theoretical pI: 9.15**

**Grand average of hydropathicity (GRAVY): -1.254**





## Results from sequence analysis:

SAA3-like sequences:

- Low hydrophobicity
- High isoelectric point
- SFLK motif

## Protein purification vs. sequence analysis:

Theoretically low hydrophobic protein that can behave as hydrophobic, but the bulk of the protein is found to be hydrophilic

Molenaar et al. *Serum Amyloid A3 from Bovine Mammary Tissue; Progress Toward its Function and Purification*. 7th International Acute Phase Protein Congress Barcelona. 2nd October 2008

### **Teoretical properties**

**MW – 12.7 Kd monomer**

**Isoelectric point – pI 9.56-9.59 monomer**

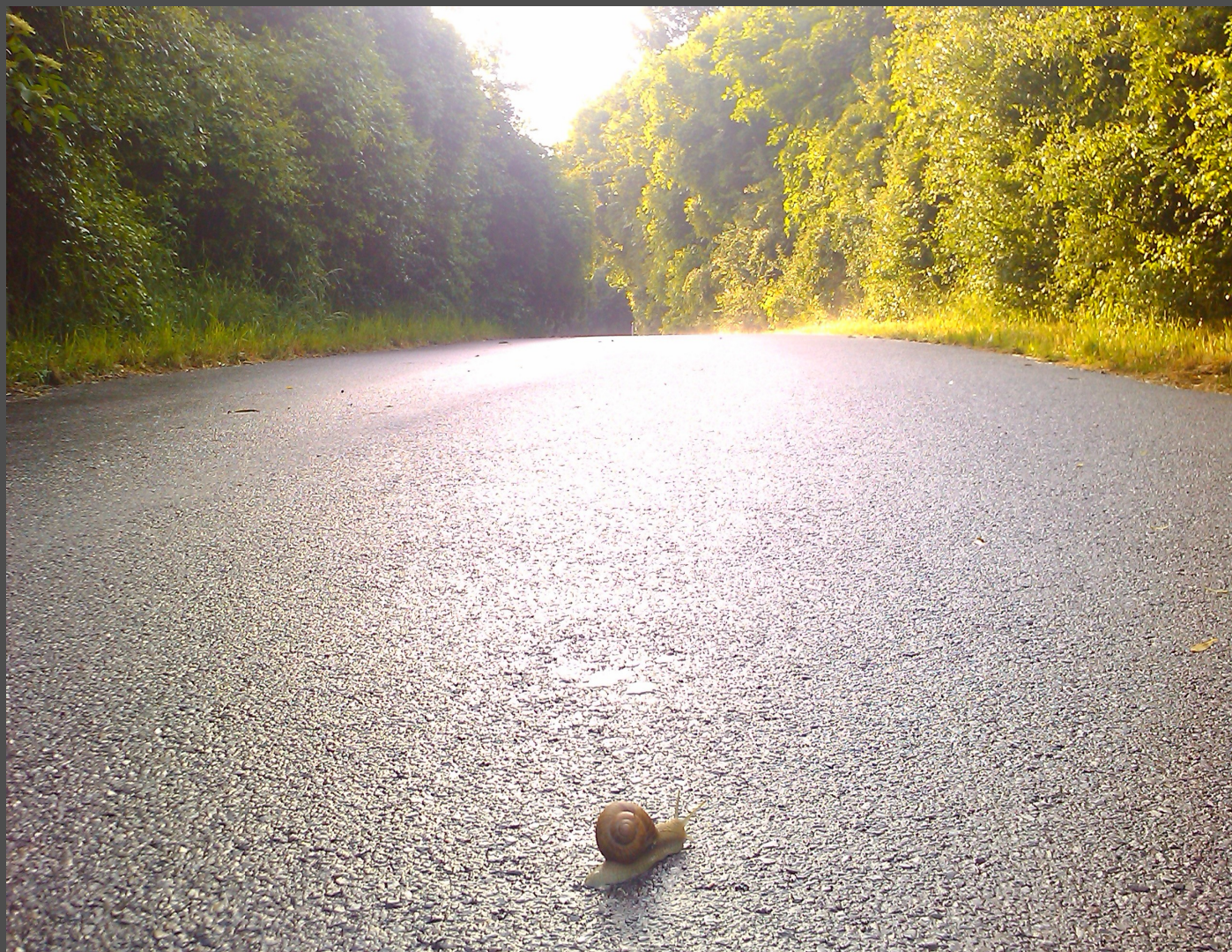
**Average low hydrophobicity**

### **Evidenced properties**

- Behaves as if it were highly hydrophobic – binds strongly to hydrophobic columns
- When purified from milk, the bulk of SAA was found in skimmed milk, not in cream. HIC not successful to purify SAA.

## CONCLUSION

The results obtained in this study suggest that circulating SAA in the pig might be a SAA3-like protein, which would preclude its function as an apolipoprotein and would explain the high resistance of pigs to the development of AA-amyloidosis



Thank you

Kiitoksia

Gracias

Tack så mycket

Mange tak

Vielen Dank

Dank u wel

Merci

Grazie mille

Obrigado