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Puroindolines and neutrons: determining the function of a family of sticky, non-stick wheat proteins

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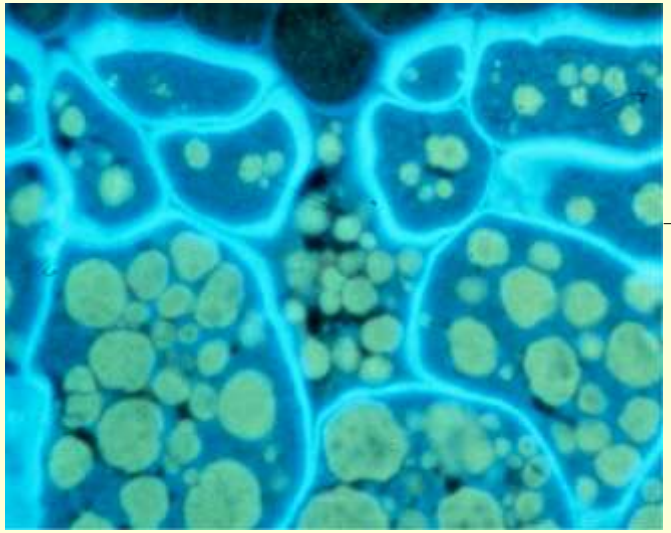
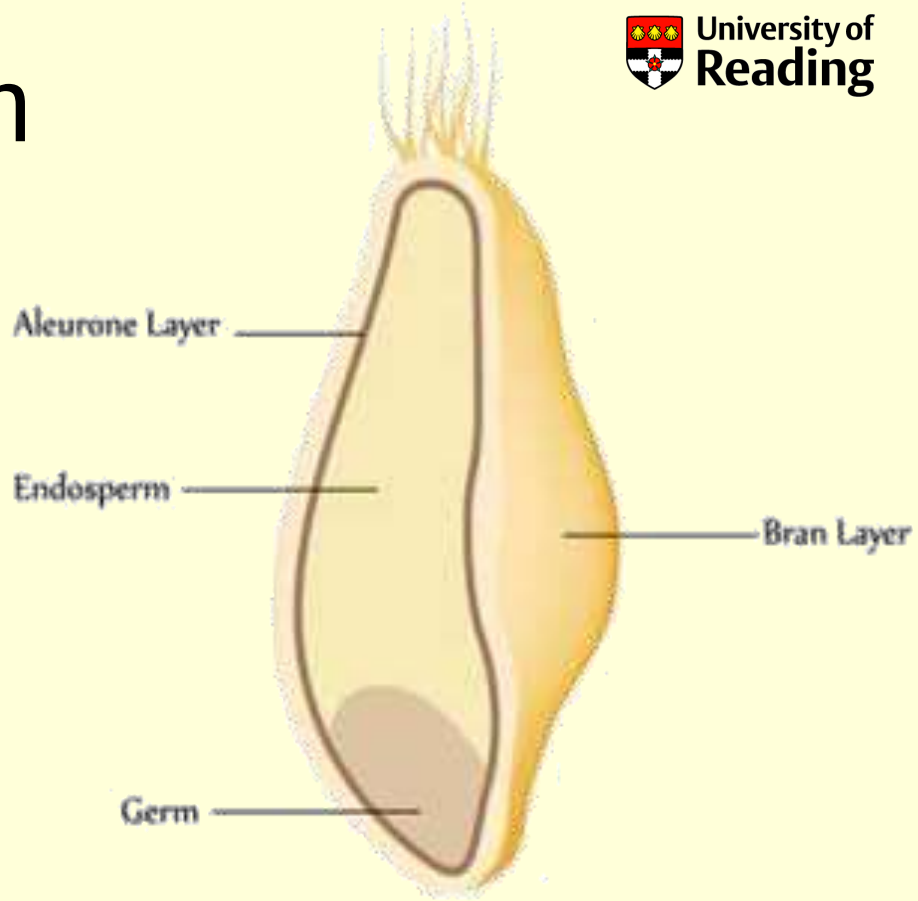
Neutrons and Food – TU Delft – 31 January 2012

Wheat endosperm





- Starch granules embedded in protein matrix
- Texture determines milling quality
  - major quality parameter of wheat grain

Endosperm texture

SOFT **HARD**

- Starch granules are loosely attached to protein matrix
- Easy to grind
- Fine-textured flour

- Binding of starch to protein matrix is stronger
- More force needed to crush kernel
- Coarse-texture
- Higher content of broken starch granules

Puroindolines (Pins)

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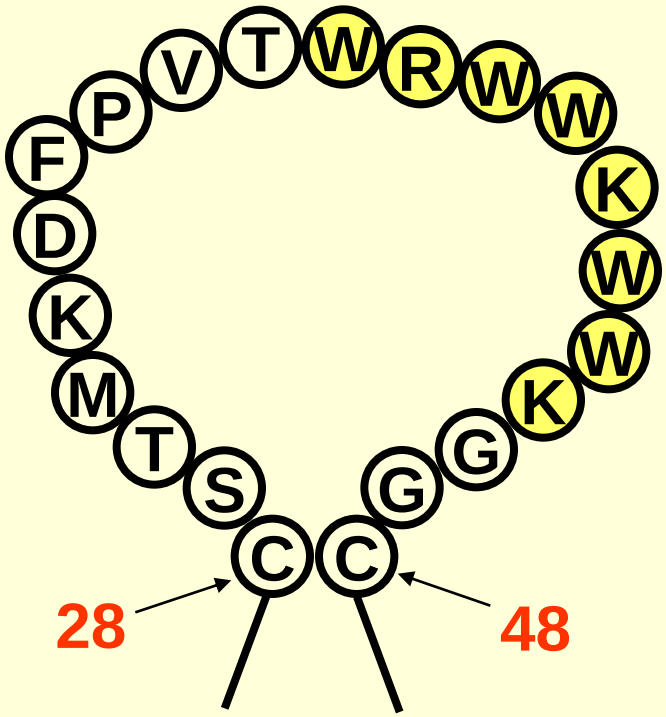
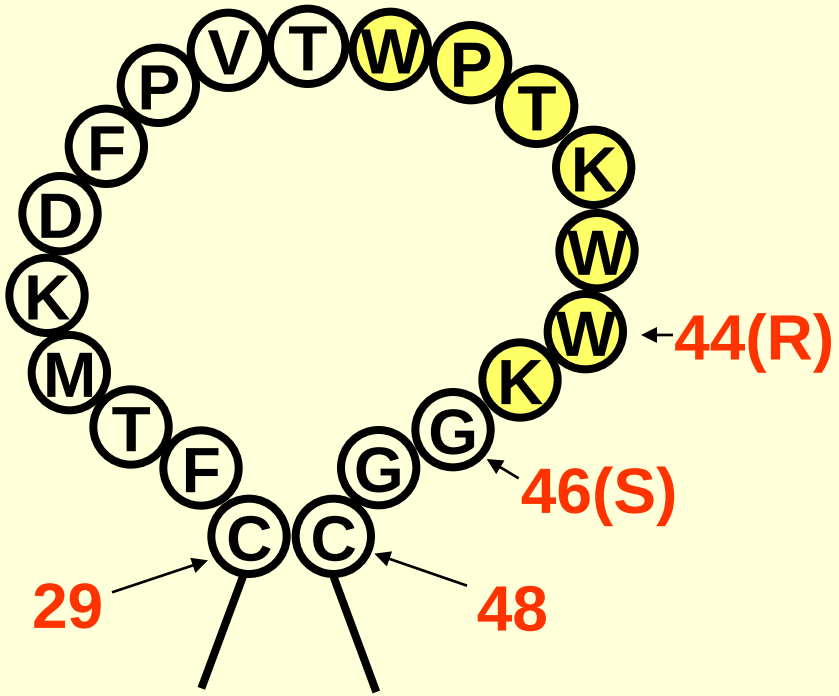
- Endosperm texture related to the binding strength between starch granules and protein matrix
- Mediated by the presence of puroindolines at this interface?
  - ‘Soft’ bread wheat: ‘wild type’ *Pin* alleles
  - ‘Hard’ bread wheat: *Pin-a* or *Pin-b* mutation
  - ‘Very hard’ durum wheat: *Pin* genes absent
- How?
  - ‘Non-stick’ coating of starch?
  - Role of lipids?

Tryptophan-rich loop/domain

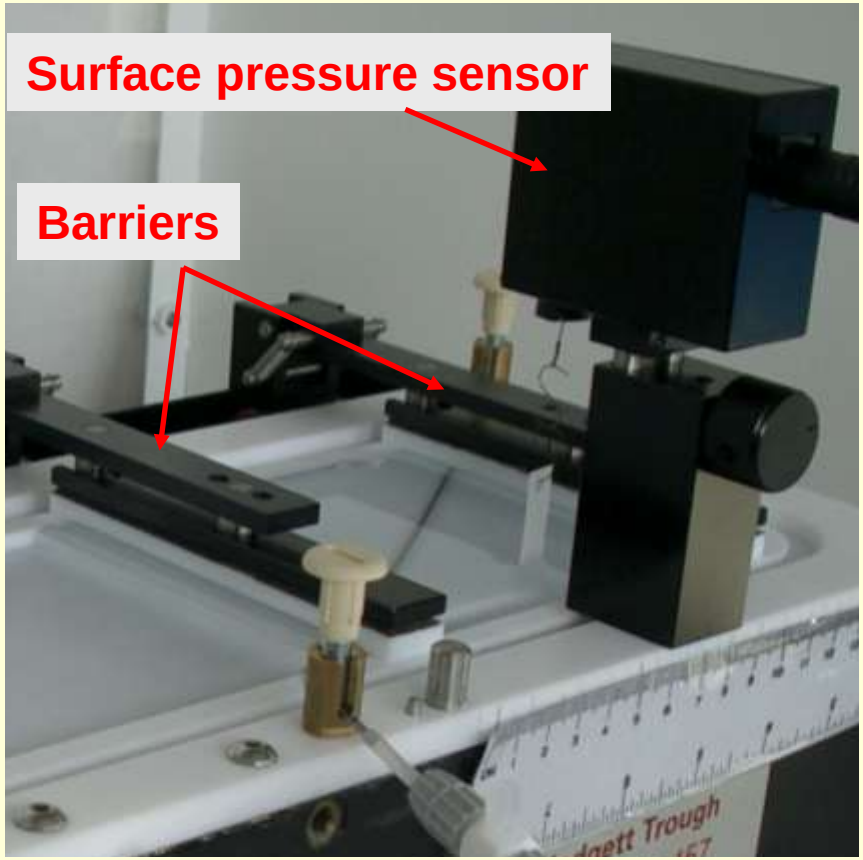


Pin-a



Pin-b

Lipid monolayer model



- Spread at air/water interface
- Compressed to liquid condensed phase
- Protein added to aqueous sub-phase
- Monitor by surface pressure isotherm, FTIR spectra, Brewster angle microscopy, neutron reflectivity

Lipid compression isotherms

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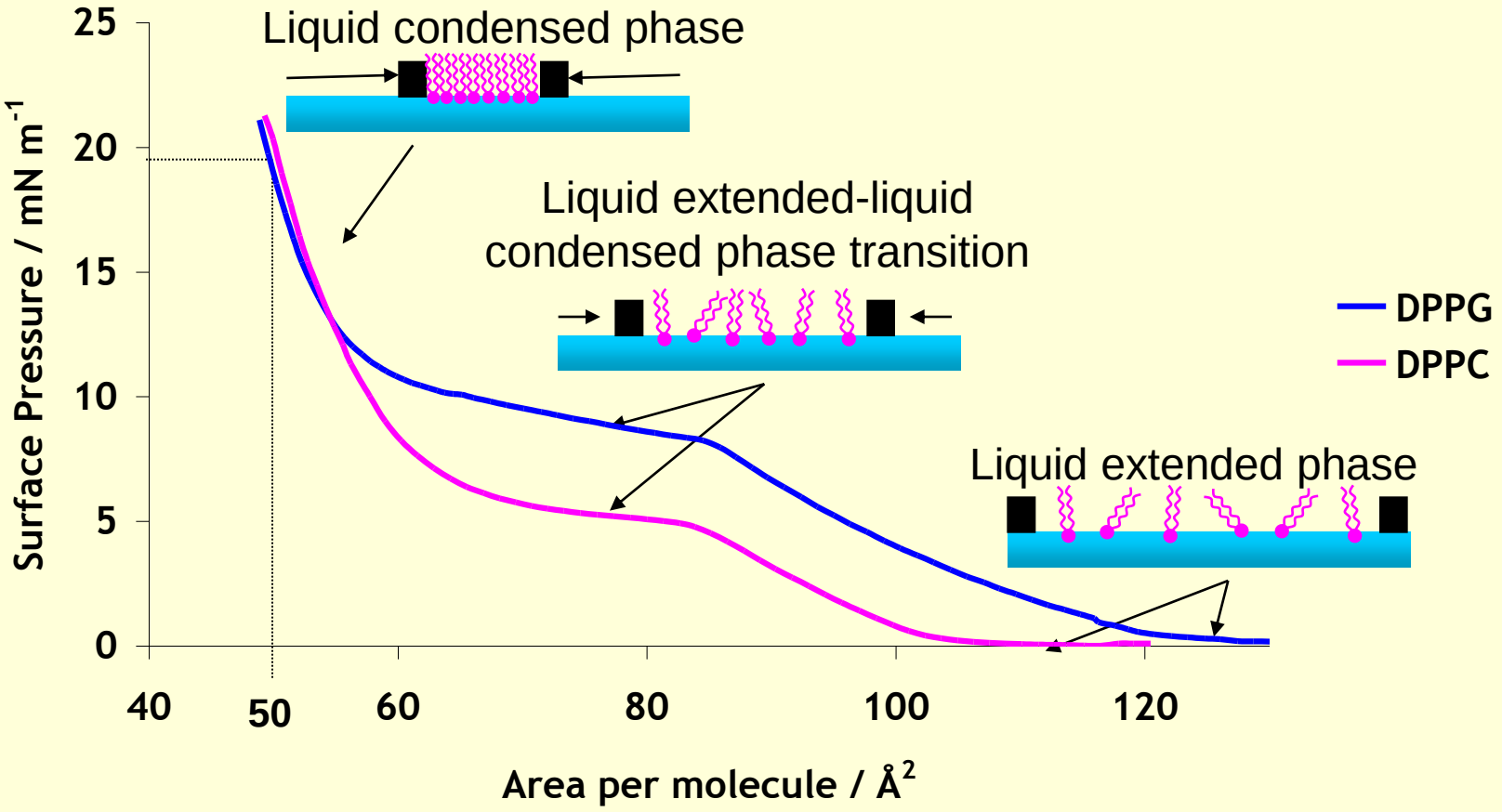

Pin-a and Pin-b

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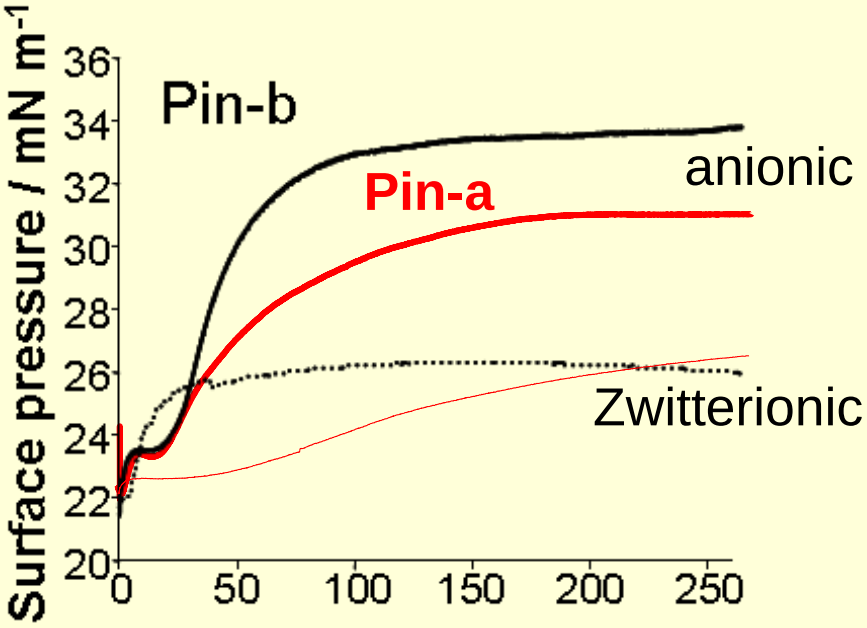

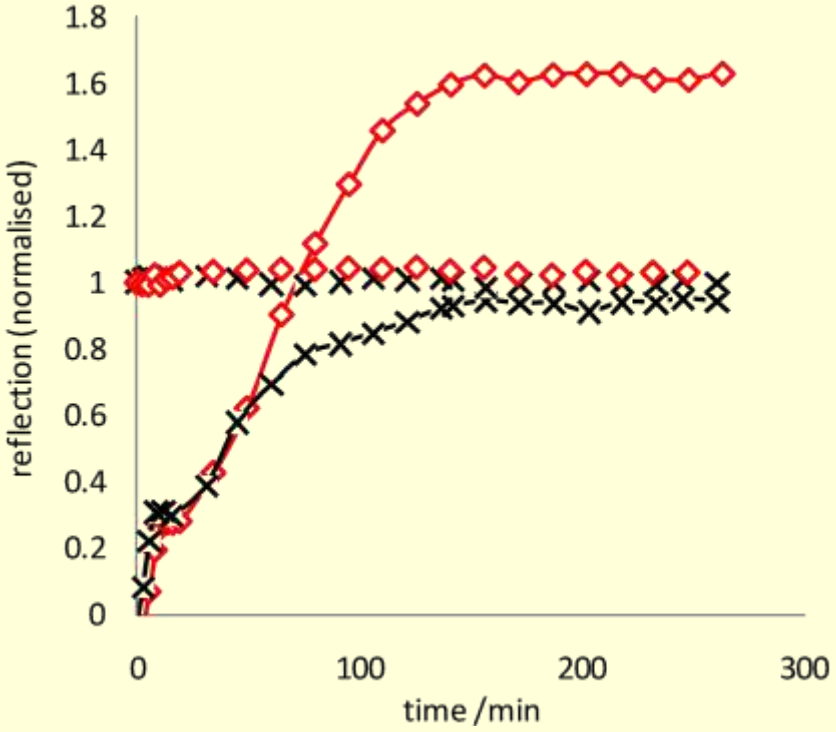

Pin-a to DPPG

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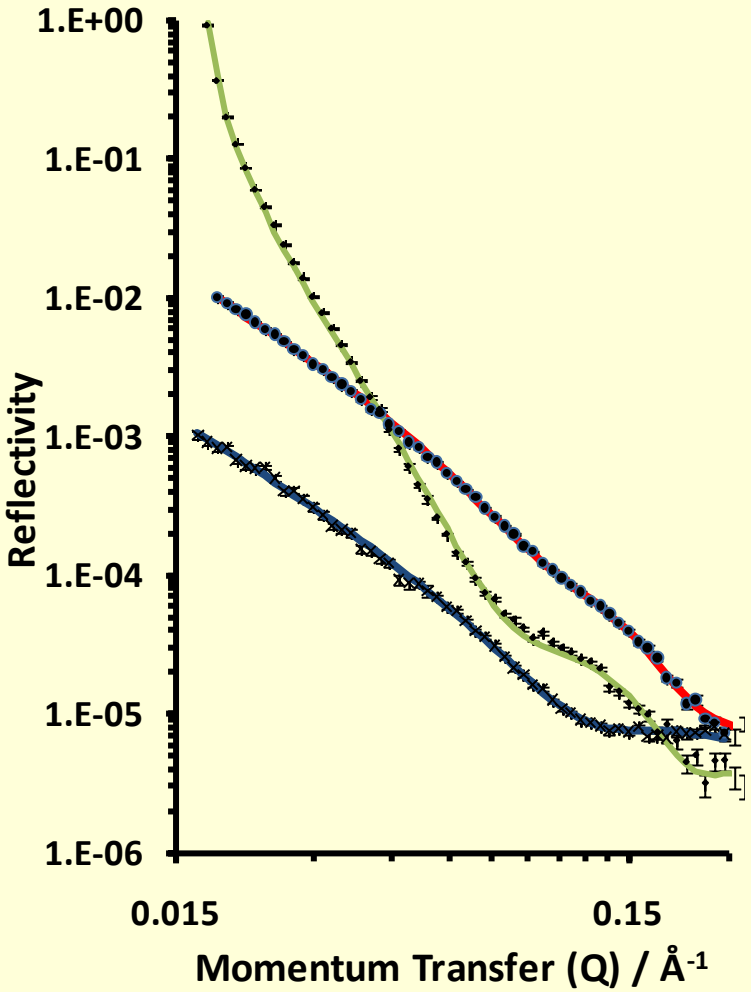
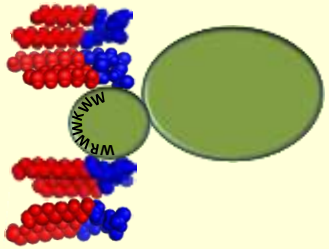
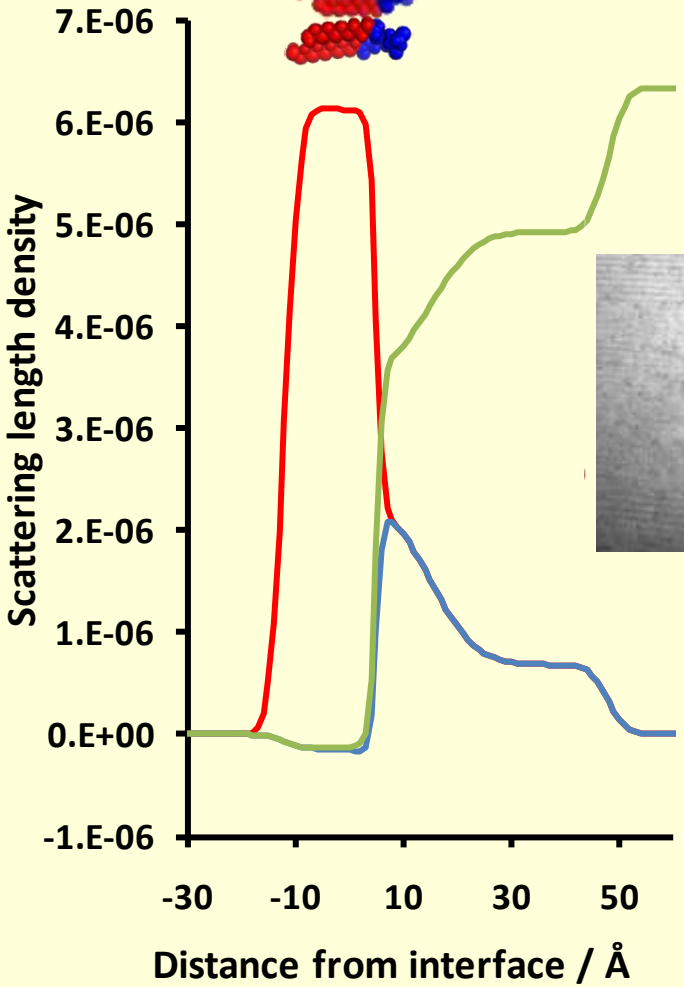
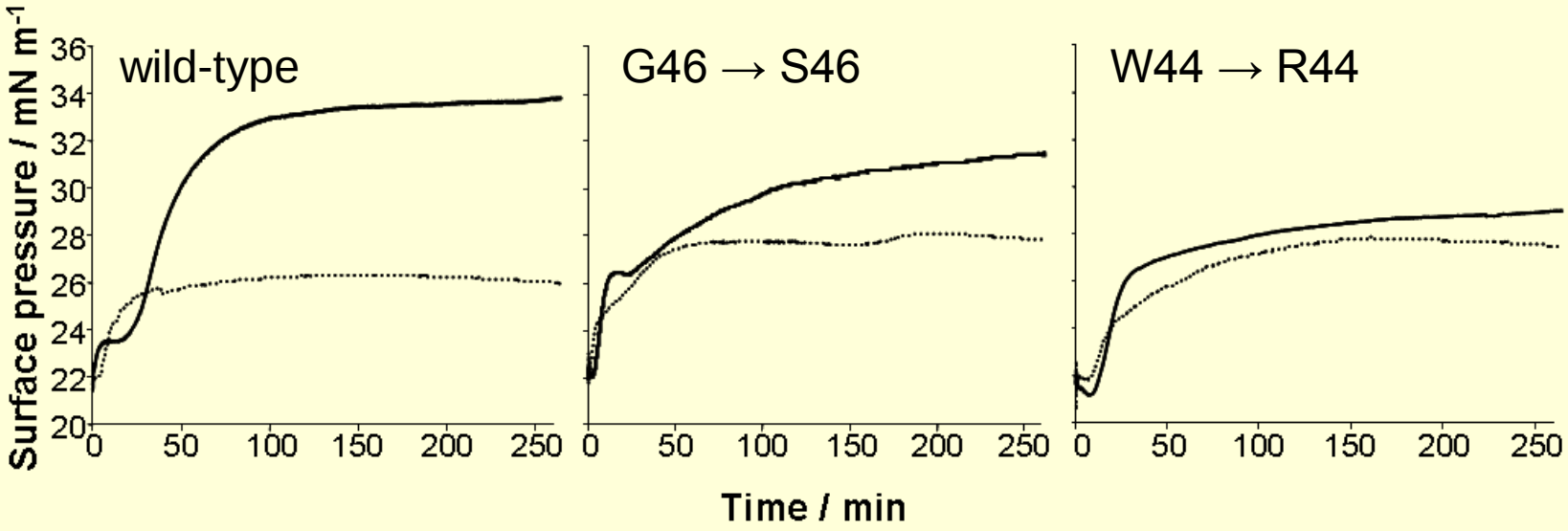






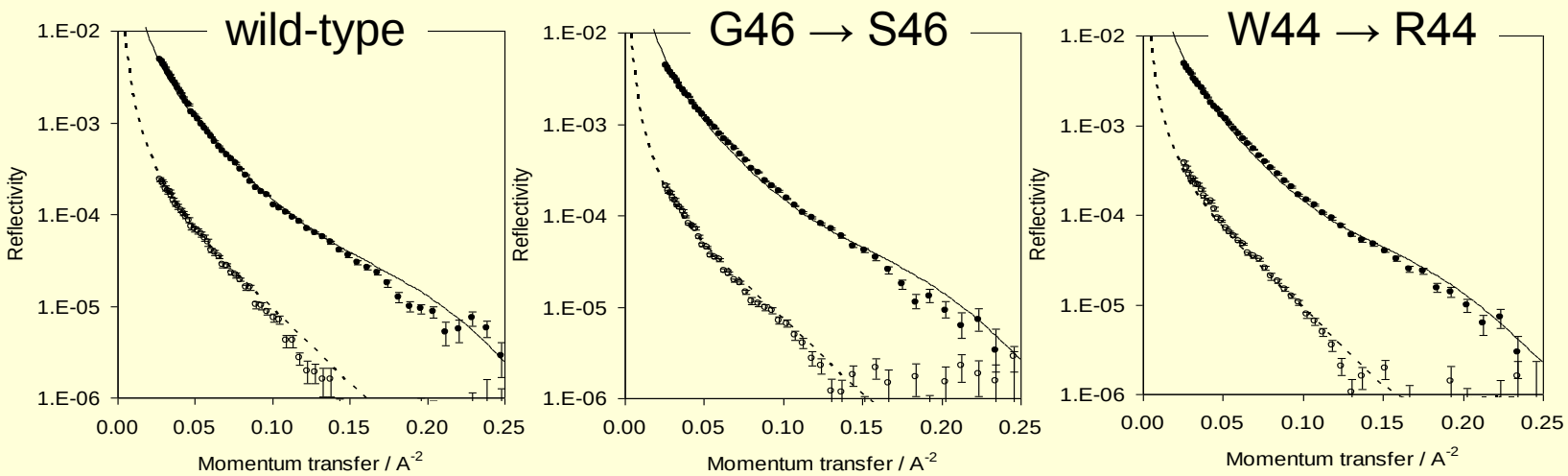
Pin-b mutants



Anionic DPPG

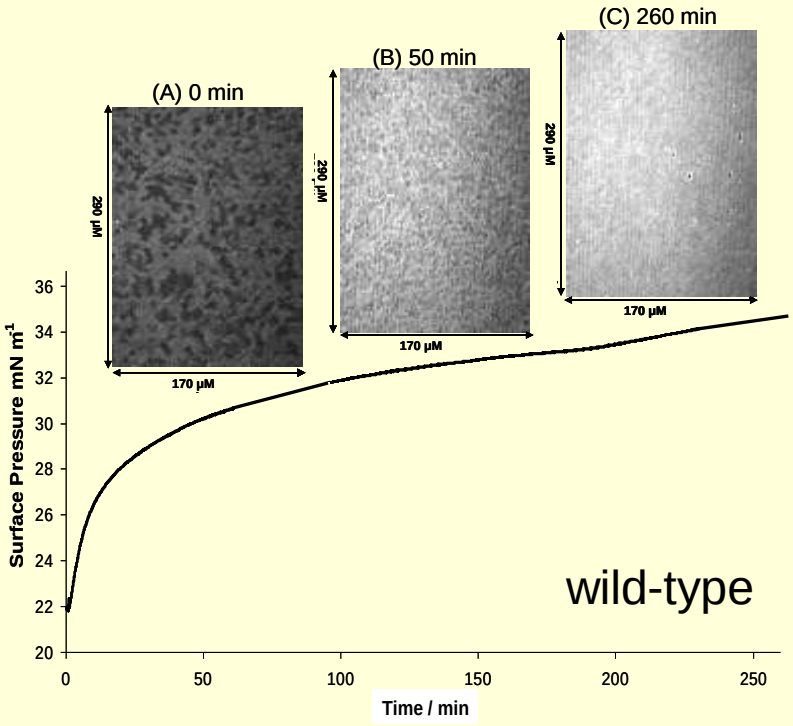
Zwitterionic DPPC

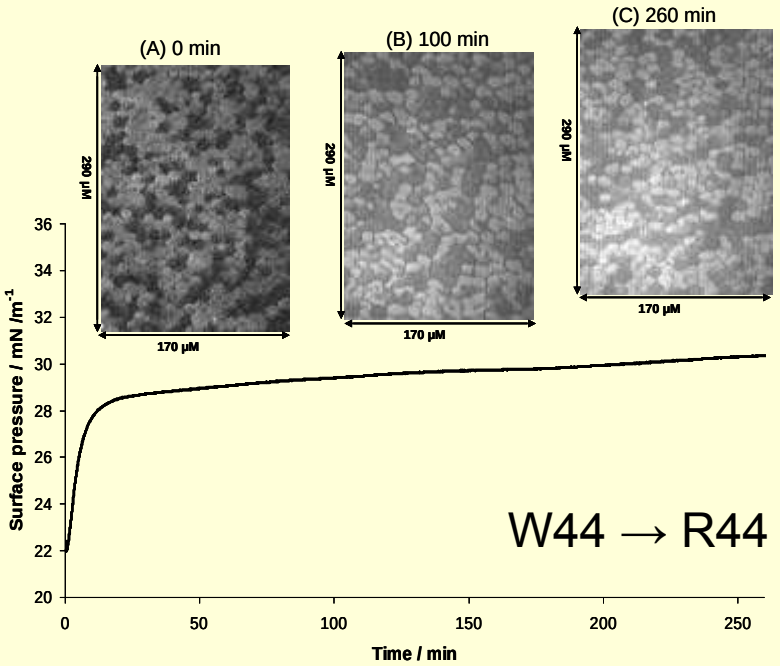
Neutron reflectometry



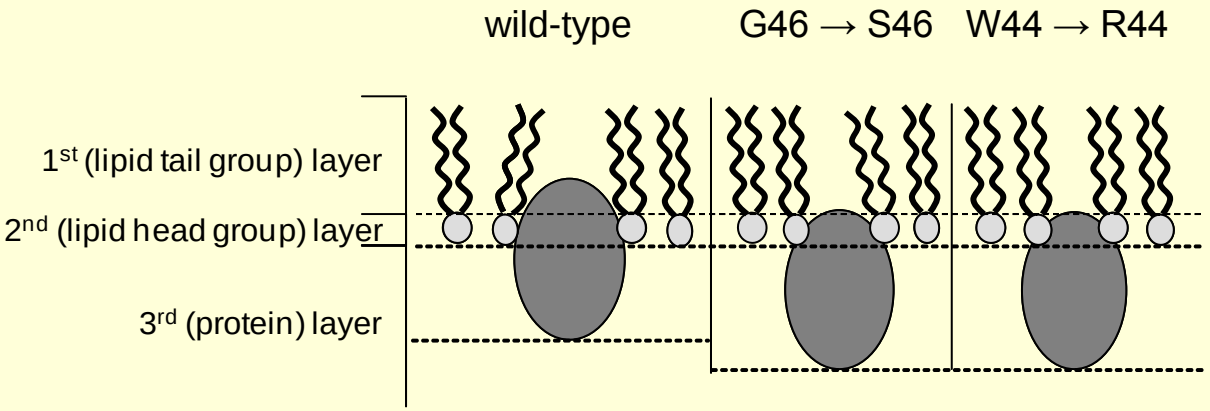
	Wild-type $\Gamma_{protein}$ / mg m^{-2}	**G46-S46** $\Gamma_{protein}$ / mg m^{-2}	**W44-R44** $\Gamma_{protein}$ / mg m^{-2}
Tail region	0.42±0.11	0.18±0.08	0.13±0.20
Head region	0.37±0.09	0.39±0.01	0.43±0.20
Below lipid region	1.27±0.15	1.58±0.18	1.3±0.2
Total protein	1.95	1.97	1.74

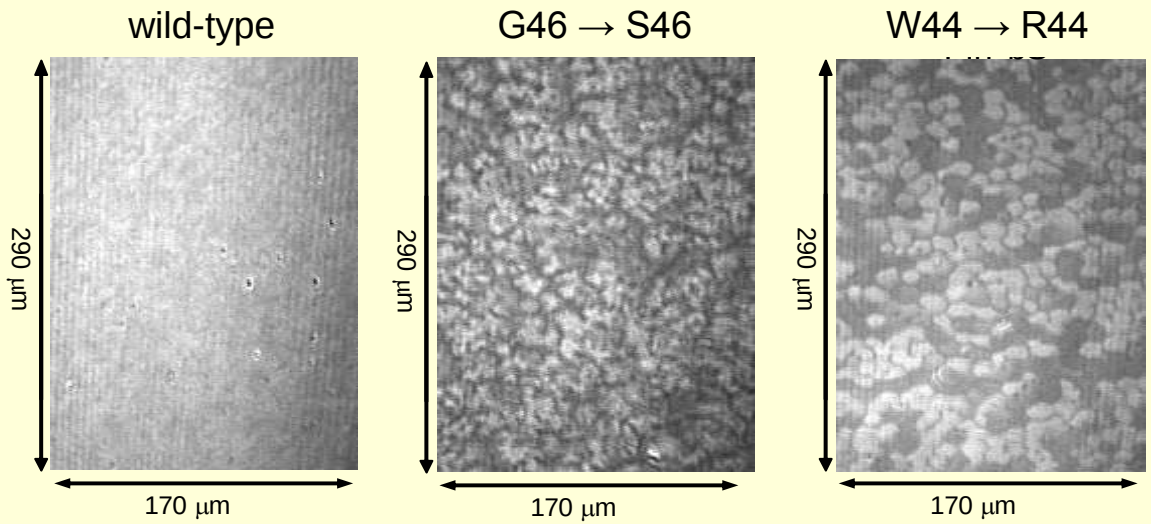
Lipid domain structure – BAM



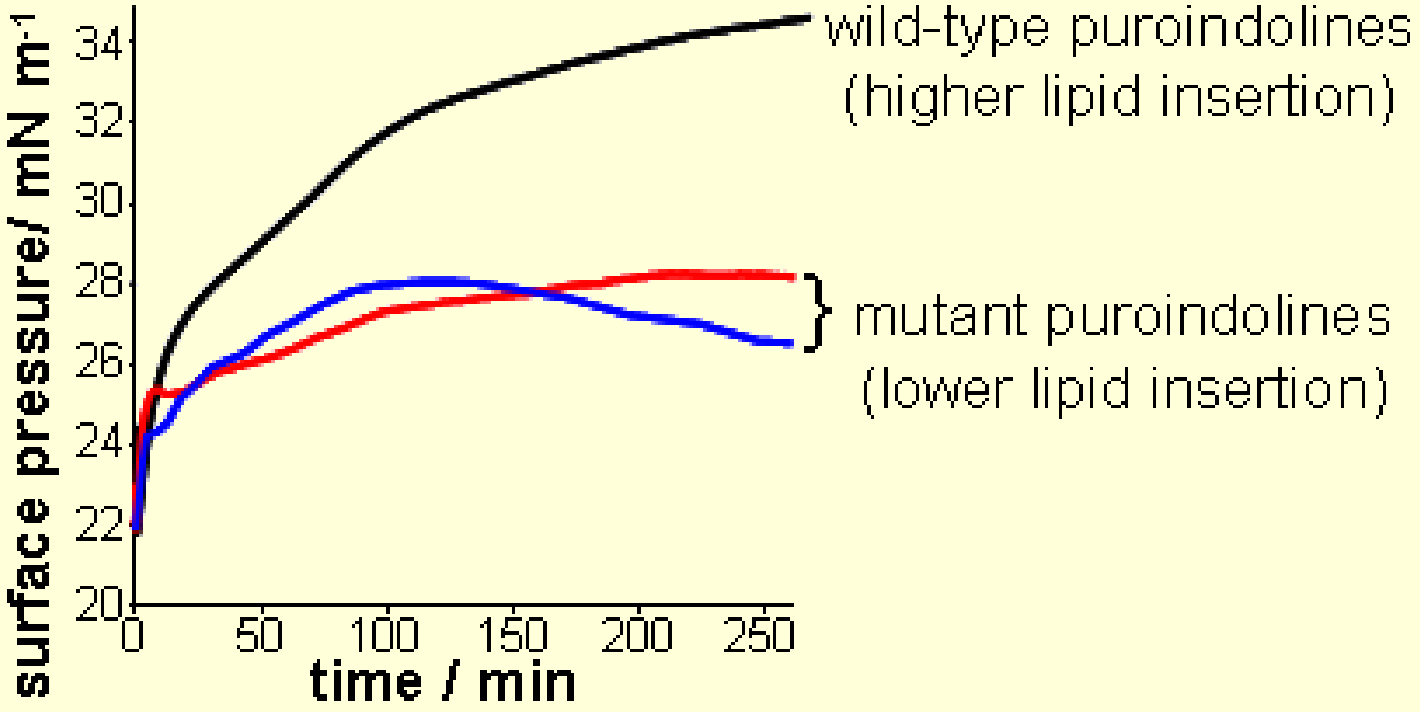


NR and BAM


wild-type
G46 → S46
W44 → R44
1st (lipid tail group) layer
2nd (lipid head group) layer
3rd (protein) layer


wild-type
G46 → S46
W44 → R44
290 μm
170 μm
290 μm
170 μm
290 μm
170 μm

Mixed Pin-a/Pin-b



Conclusions

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- Pin-b mutants show lower insertion into DPPG monolayers
- Gly46 → Ser46 introduces steric constraint on the conformation of the Trp-rich loop
- Trp44 → Arg44 replaces non-polar residue with basic residue within the Trp-rich loop
  - increases the polarity of the loop
  - increased electrostatic interaction with anionic head group?

Future: plant seed defence

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- Puroindolines are involved in defence against fungal pathogens
  - Synergistic activity with other plant defensins?
- Studying puroindoline/purothionin interactions with model biomembranes
  - Are these proteins more effective in combination?
  - How do Pin-b mutations influence this?

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DPPG monolayer

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