Puroindoline Binding to Starch and its Role in Endosperm Texture

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Background 1

- Endosperm texture determines milling quality, a major quality parameter of wheat grain
- Soft (friable) texture was correlated with ~15kDa starch granule surface protein, termed “friabilin”.
- Presence of friabilin controlled by *Hardness* gene on chromosome 5D short arm
- Friabilin later characterised as puroindolines (“PINs”), very basic proteins
- PIN protein soft alleles remain bound to starch granules as friabilin after water washing
- Hypothesis: friabilin acts as “non-stick” protein

Background 2

Puroindolines

- low M_r , 13kDa non-gluten proteins
- cysteine-rich (5 S-S bonds), very basic (pI > 10)
- folding similar to wheat nsLTP with extra inserted amphiphilic loop (Trp domain, up to 5 Trp)
- highly surface active
- polar lipid binding and foam stabilising properties

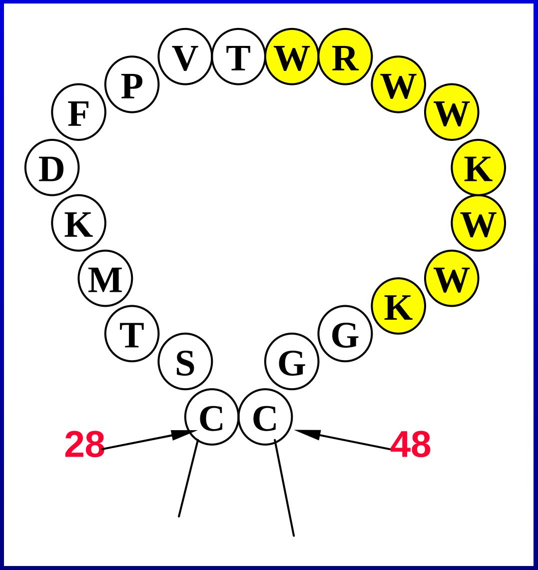
Background 3

- ***Pin*** **genes absent in durum wheat (tetraploid AABB genome)**
- **Breadwheats (AABBDD) when soft always have the soft "wild type"** ***Pin*** **alleles, as when inserted with** ***T. taushii*** **(DD) parent**
- **Hardness in breadwheat is associated with later mutations of** ***Pin-a*** **or** ***-b***
  - ***Null*** **mutations of either** ***Pin-a*** **or** ***Pin-b***
  - **Point mutations in** ***Pin-b***

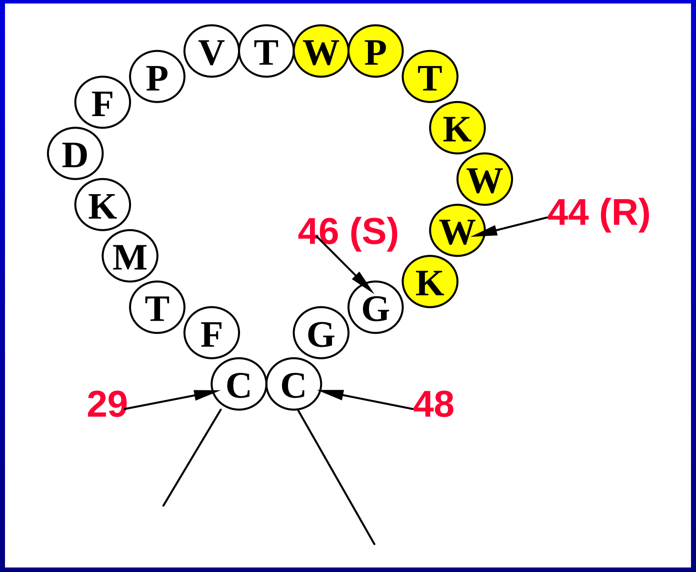
Gly → *Ser,*	residue no.	46
Trp **→** ***Arg,***	**"**	**44**
Leu **→** ***Pro,***	**"**	**60**
Lys **→** ***Glu,***	**"**	**45**

Amino Acid Substitutions within the Tryptophan Loops of PINs

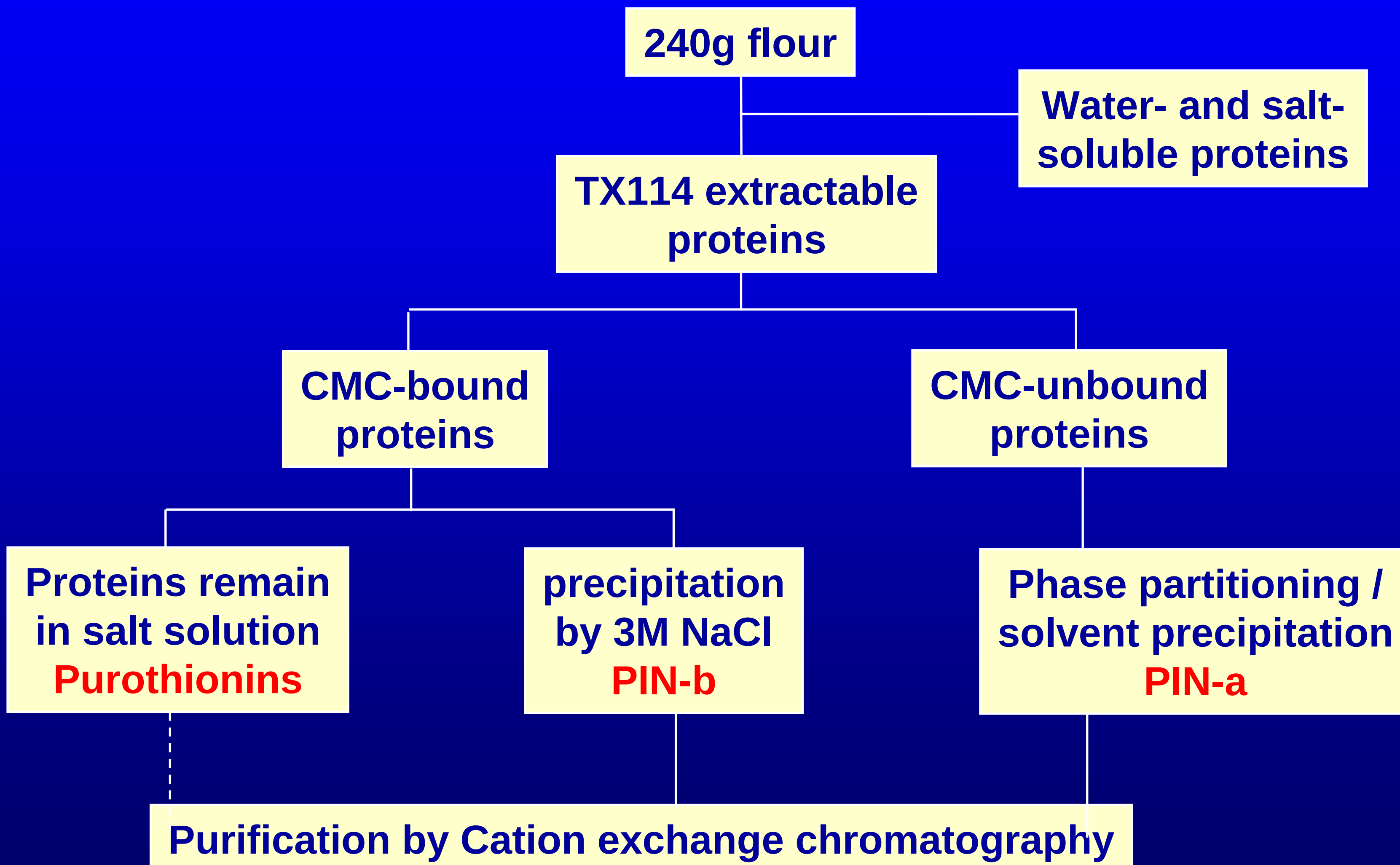
Puroindoline-a



Puroindoline-b

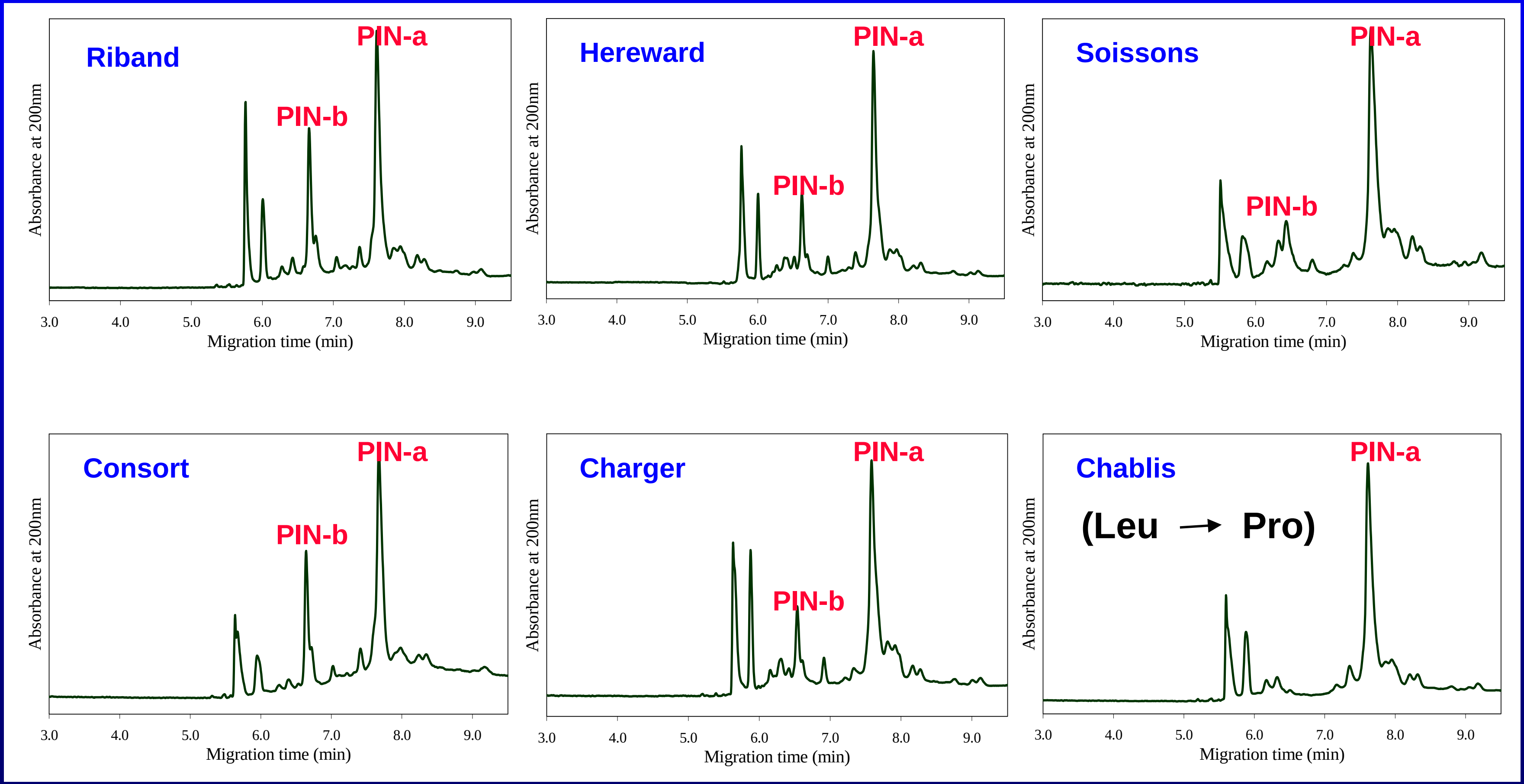


Extraction of PINs by TX114 and CMC adsorption

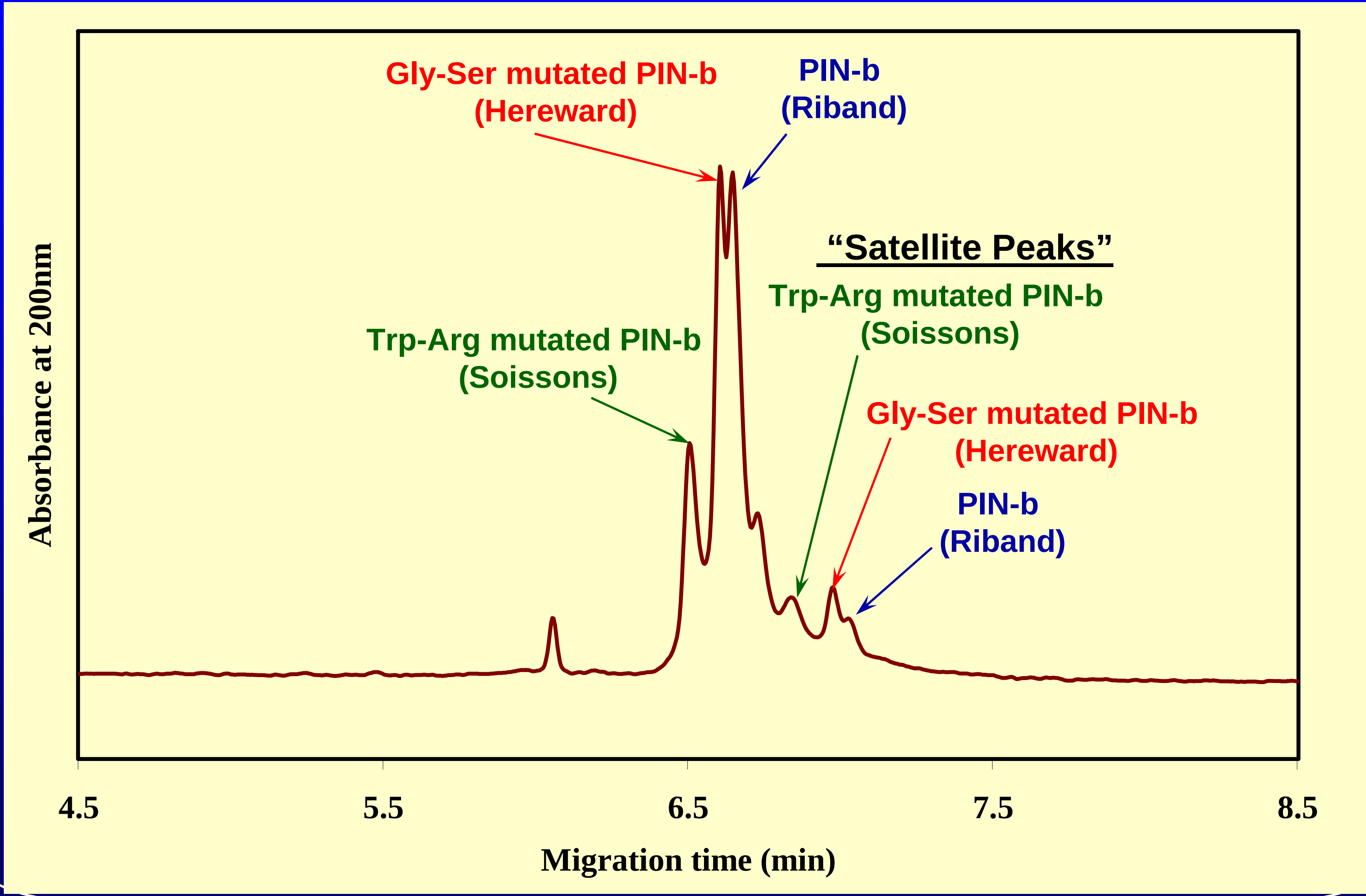


CE Profiles of TX114 Extracts
- Small-Scale Extraction

Soft, wild type	Hard, Gly → Ser	Hard, Trp → Arg

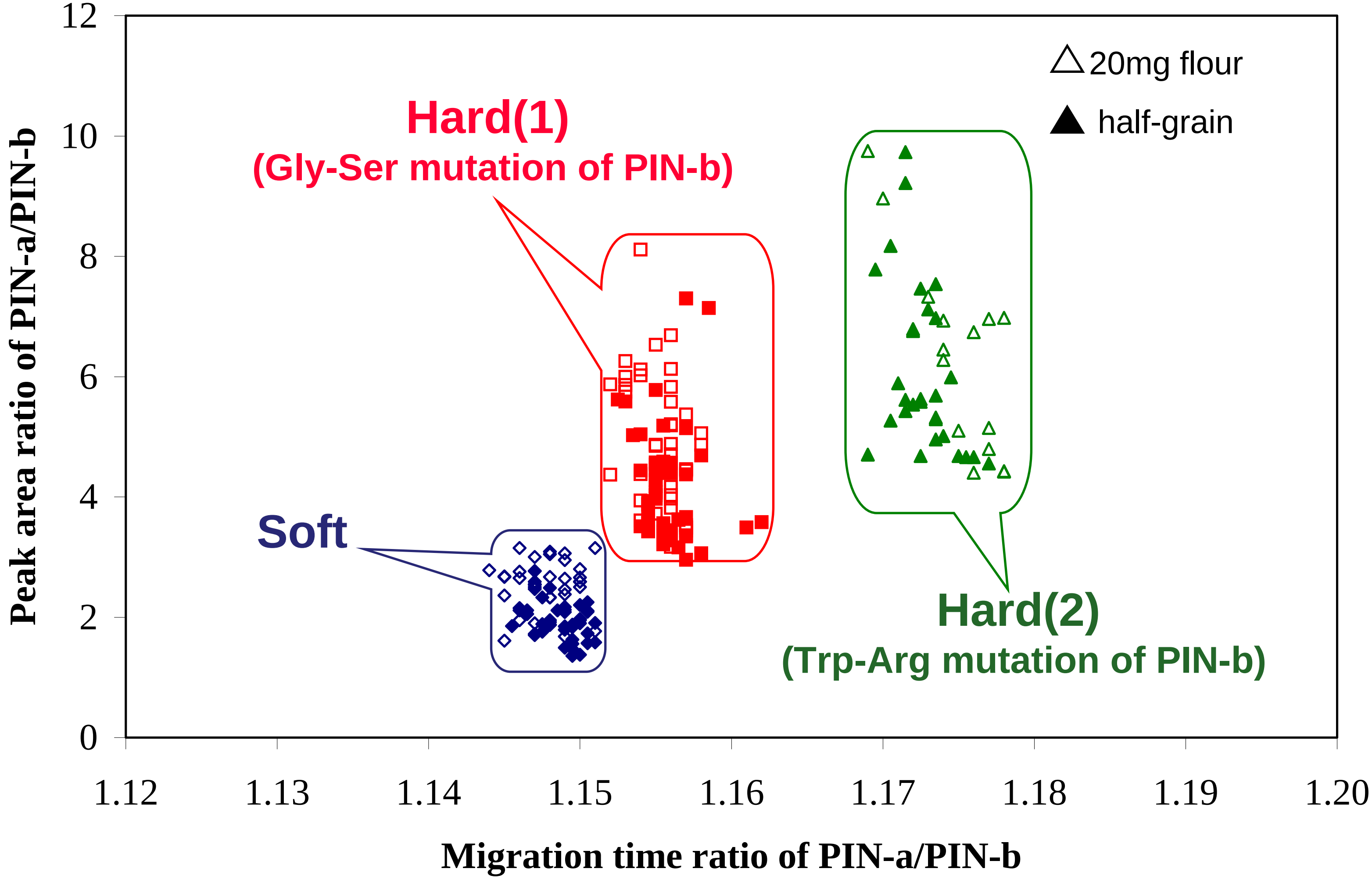


CE profile of a mixture of three purified PIN-b proteins



C C F R A

Grouping of wheat types based on CE characteristics



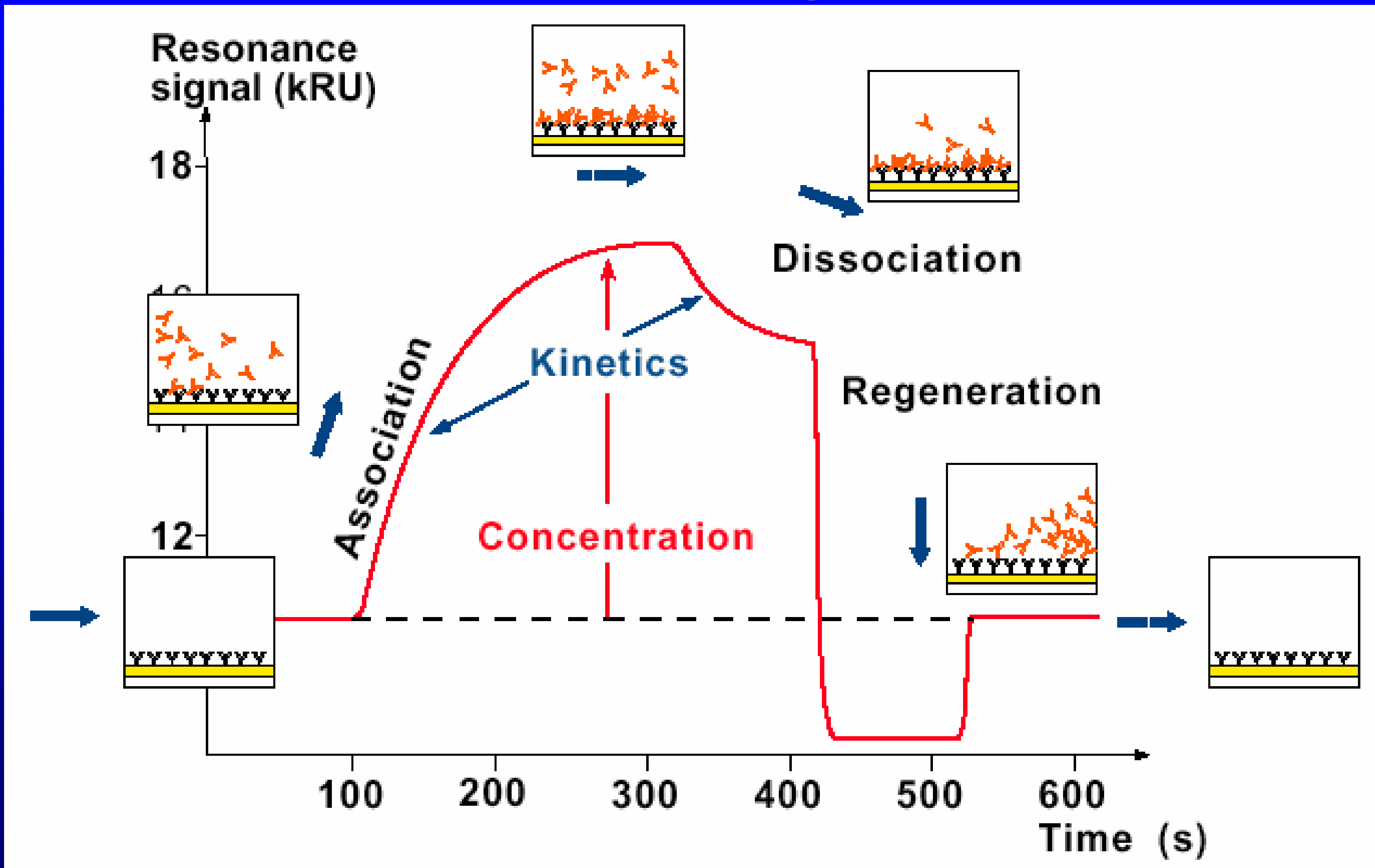
Summary 1

- Purification method for PINs developed, based on CMC adsorption/TX114 phase partitioning.
- Structural studies of purified PINs from wheats with different endosperm texture.
- Allelic forms of PIN-b were found and characterised by CE and Mass Spectrometry.
- Analytical method developed for half-grains that allows rapid examination.
- This could be used to screen for endosperm texture of lines during breeding programmes.

Kinetic measurement of PIN binding to a Model Starch Surface using

Surface Plasmon Resonance

The Sensorgram

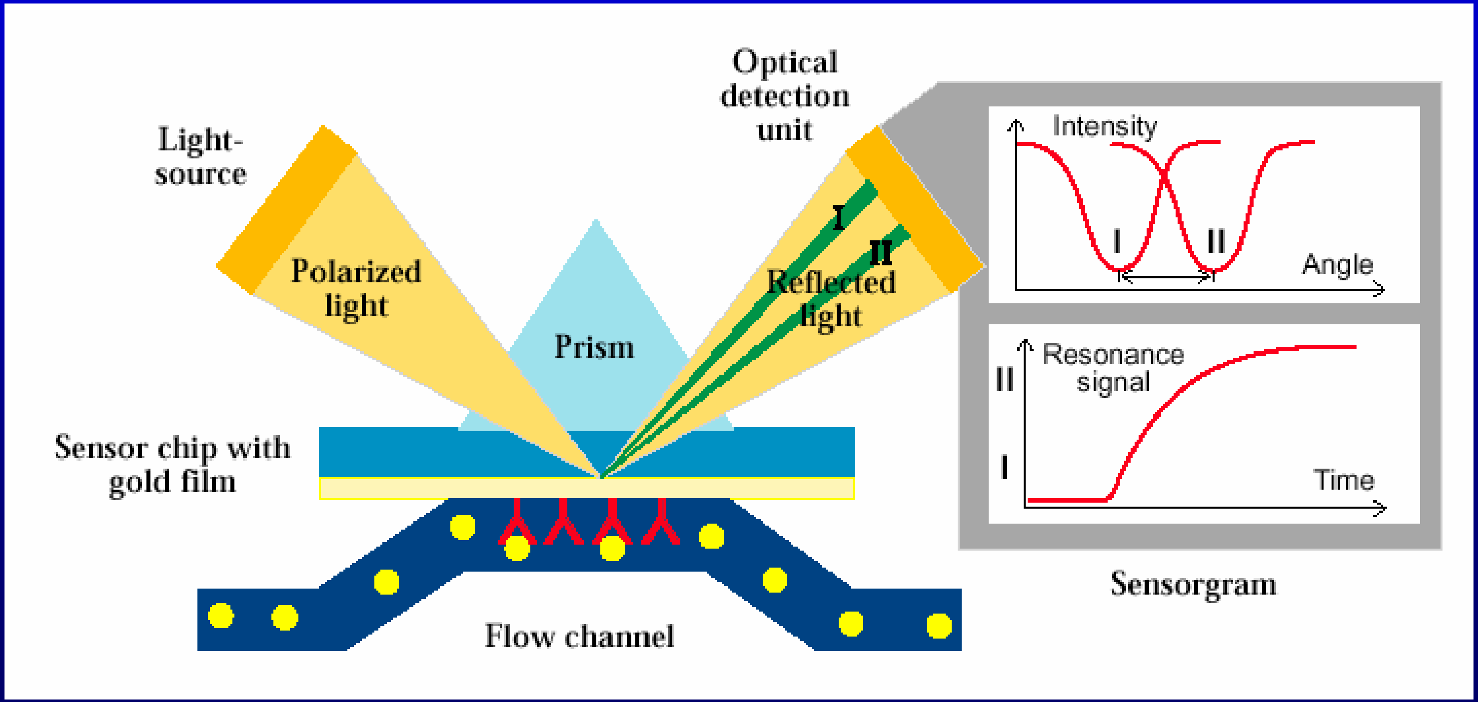


How does BIACore Measure this?

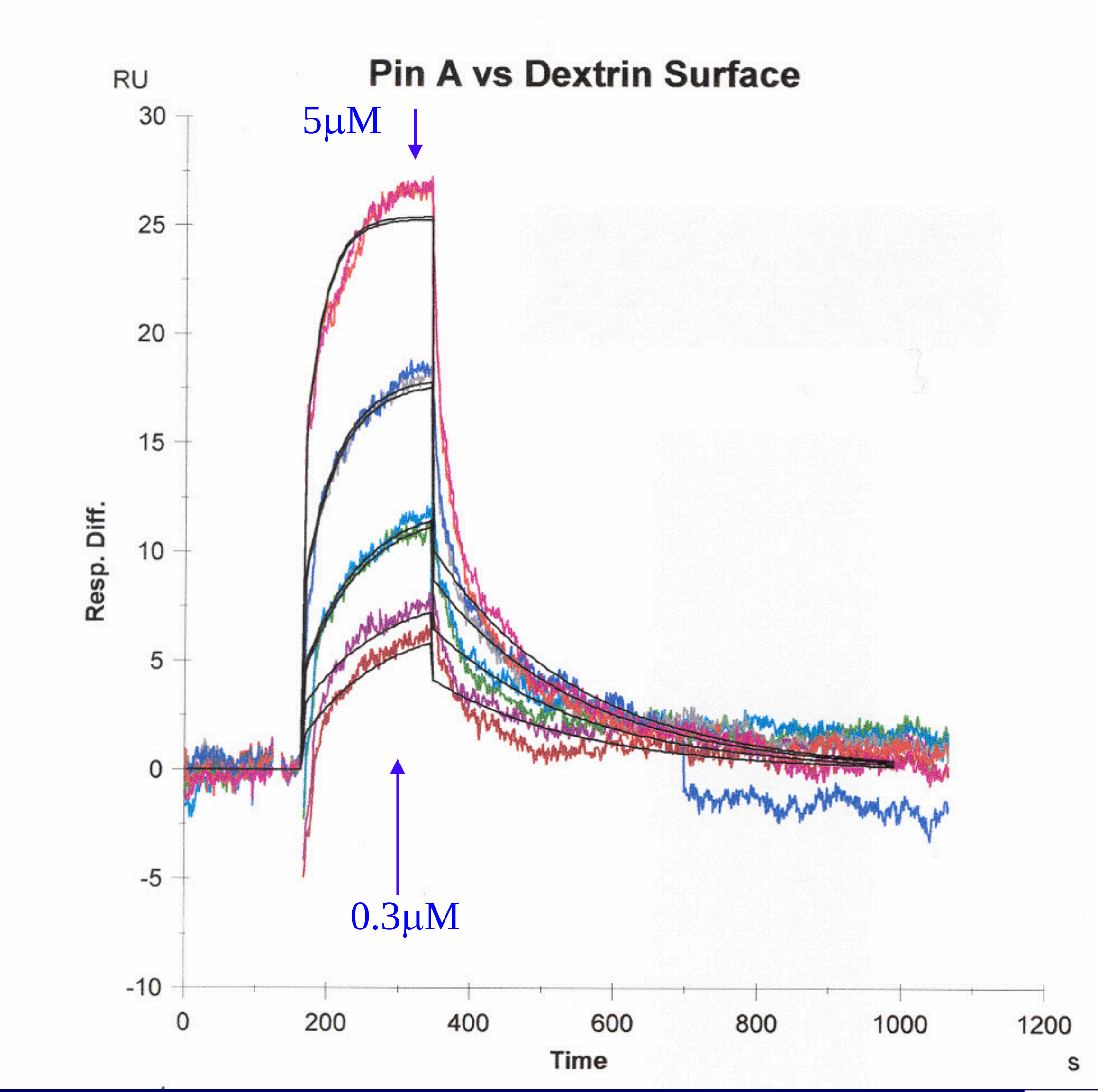
He-Ne laser (λ = 632.8 nm) focused on gold surface film, gives dark band at internal reflection angle of photon/electron resonance (“surface plasmons” dissipate energy)

Ligand binding at surface gives **change in resonance angle,** by altering **refractive index** seen by evanescent wave that penetrates surface medium (~500-600 nm)

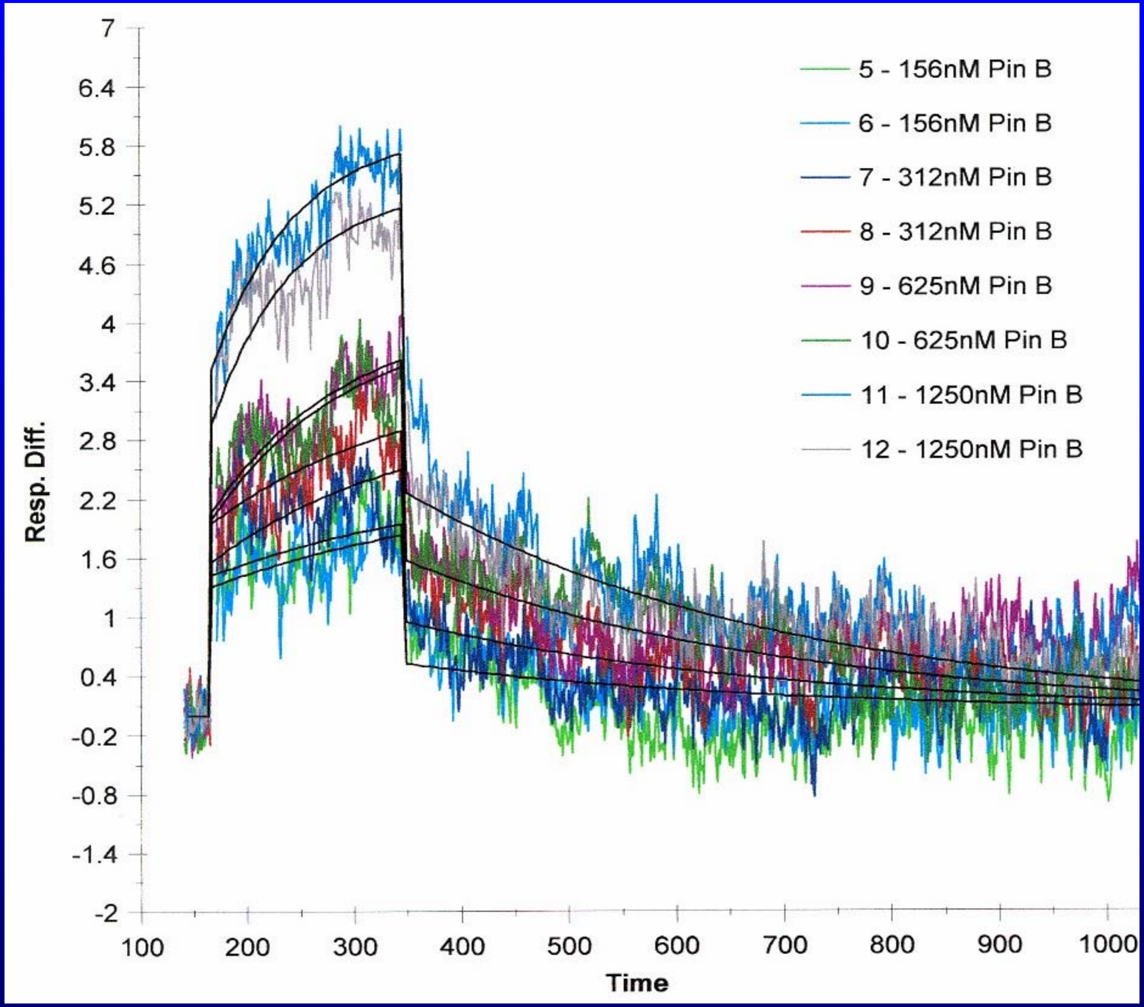
I - Unbound state   II - Bound state



PIN-a Binding Curves



PIN-b Binding Curves



Summary of PIN Binding (Clare, a Soft-milling Wheat)

Global Fit Binding Model

	KA (M^{-1})	KD (M)	R_{max} (RU)	Chi^2
Pin A (crude)	1.66×10^6	6.01×10^{-7}	7.28	1.08
Pin A	2.64×10^6	3.78×10^{-7}	11.9	0.76
Pin B	2.91×10^6	3.44×10^{-7}	3.19	0.12

Conclusions

- Study has demonstrated specific binding interaction between PINs and amylodextrins - a model for the starch granule surface
- This can be studied in a quantitative manner by SPR
- Binding constants for PIN-a and PIN-b were similar
- Amount of bound PIN-b less than third that for PIN-a
- May indicate conformational difference when bound
- Binding supports idea of non-stick coating on granule?

Proposed Studies on Role of PINs

- Further SPR binding studies with purified PINs
  - synergy between wild-type PIN-a and PIN-b?
  - binding of hard allelic forms of PIN-b
  - chemical modification of Trp or Arg in Trp domain
  - with 1- and 2-chain dextrins, & other glycans
  - the influence of polar lipid in interactions
- Further binding studies on PINs
  - atomic force microscopy
  - isothermal titration calorimetry
  - spectroscopy
- Protein characterisation of PIN b variants
  - 2D PAGE, MS, CD, FT-IR, NMR

PINs in "high-ratio" cake baking – a LINK opportunity?

- Native 'wild type' PINs are known to control the physical chemistry (adhesion) at the starch granule surface
- Increased hydrophobicity at starch surface plays a critical role in known "high-ratio" treatments of flour for cakes (chlorination, acetylation, or heat/moisture)
- Proposed work will extend CCFRA hypothesis, that treatments function mainly by denaturing PINs
- Objective is to devise improved physical flour treatments via improved mechanistic understanding
- This will benefit cake flour manufacturers and bakers through improved production and performance of "clean label" flour