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Corneal Dermato-pontin Interacts with Keratan Sulfate Proteoglycans

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Abstract

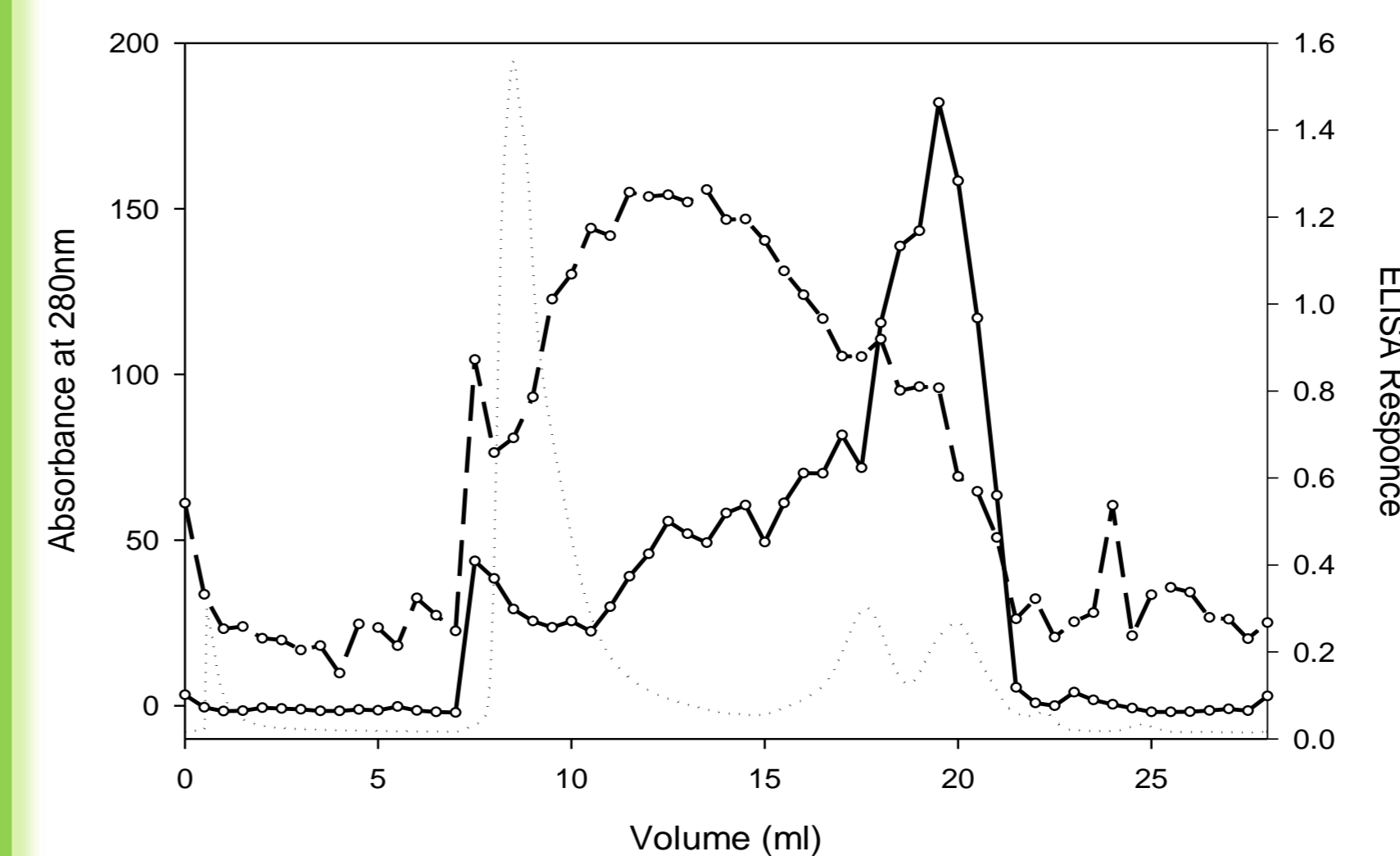
Dermato-pontin is an extracellular matrix protein with many functions, amongst which is the involvement in collagen fibrils' organization. It has been isolated from bovine corneal stroma, alone and in a complex with keratan sulfate proteoglycans, using 7 M urea followed by ion-exchange chromatography. Chromatographic and antibody-based studies suggest that dermato-pontin binds to lumican and keratocan keratan sulfate proteoglycans (KSPGs), and that it binds directly to corneal keratan sulfate chains. As the protein cores of KS-PGs are known to bind collagen, our results suggest that the KS chains act as spacers between DPT and proteoglycans, and hence collagen fibrils. This would contribute to the pattern of organization of the fibrils that is required for corneal transparency, and may explain why loss of keratan sulfate chains would result in disorganization of the collagen fibrils in the corneal stroma leading to opacity and blindness, and may also explain the effect of knocking out the *DPT* gene in mouse, resulting in disturbing the collagen organization and increase in the inter-fibril distance.

Corneal Molecular Biology

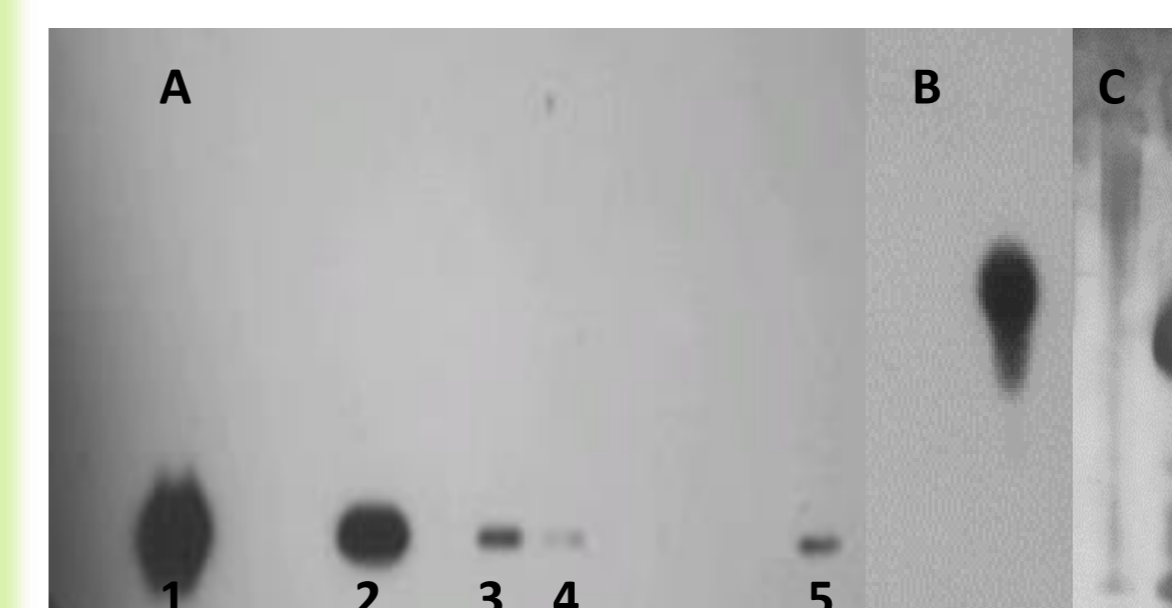
- Corneal transparency is essential for its function.
- Key to this transparency is collagen fibrils lamellae of uniform diameter and inter-fibrillar spacing.
- Major proteoglycans (PGs) in the corneal stroma are lumican, keratocan and mimecan/osteoglycin (keratan sulfate (KS) PGs), and decorin (chondroitin sulfate / dermatan sulfate (CS/DS) PG).
- Protein cores of these PGs bind collagen, and control fibril diameter.
- Keratan sulfates and their sulfation rate are essential for transparency.
- keratocan-null mice have transparent but thinner cornea, with thick fibrils and less-organized packing.
- Lumican-null mice have opaque corneas with thick fibrils and perturbed interfibrillar distances.
- Dermato-pontin (DPT) is a tyrosin-rich 22kDa extracellular matrix protein. In a previous study by our group, a disturbance of the collagen fibrillar organization and an increase in the interfibril distance compared with wild type, indicated an important role in the maintenance of corneal stromal architecture, hence corneal transparency.

Results

- Dermato-pontin is present abundantly in cornea.
- Dermato-pontin interacts with KS-PGs.



Gel-filtrations of the complex sample. (isolated from Q-Sepharose column). Absorbance was measured on-line at 280 nm (dotted line). Fractions were analysed by ELISA for dermato-pontin (solid line) and KS (5D4) (dashed line).



Western blotting results of DPT-KSPGs complex sample, after gel-filtration purification.

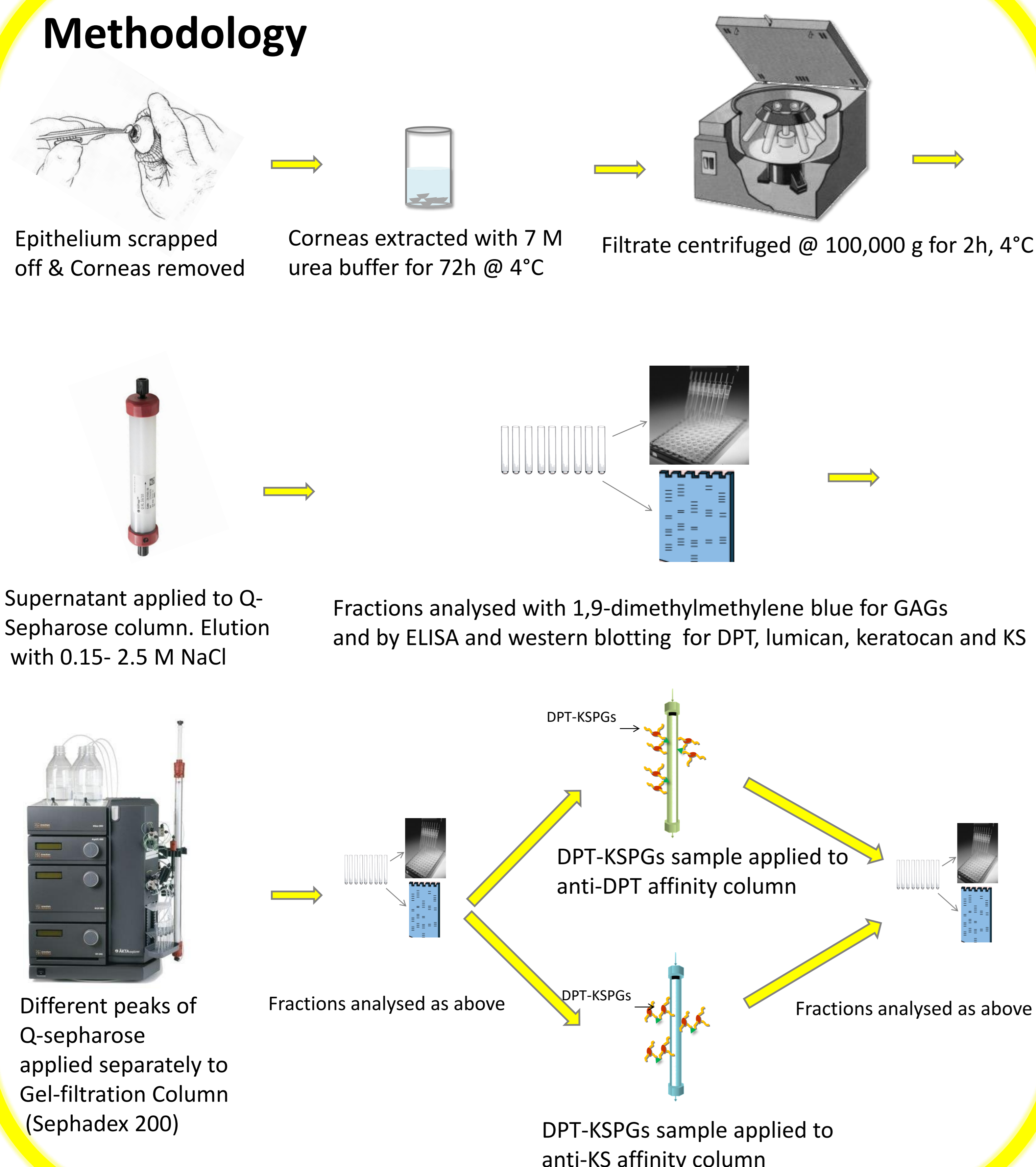
A DPT response: 1: Pure DPT. 2: DPT-KSPGs complex sample from ion-exchange column, prior to loading to gel-filtration (large peak for DPT ELISA response, see above figure). 3 & 4: DPT eluted from the two other small peaks of DPT on gel-filtration (see above figure). 5: Free DPT from ion-exchange column.

B: Lumican response of DPT-KSPGs complex sample (sample of lane 2 (A)).

C: Keratocan response of DPT-KSPGs complex (sample of lane 2 (A)).

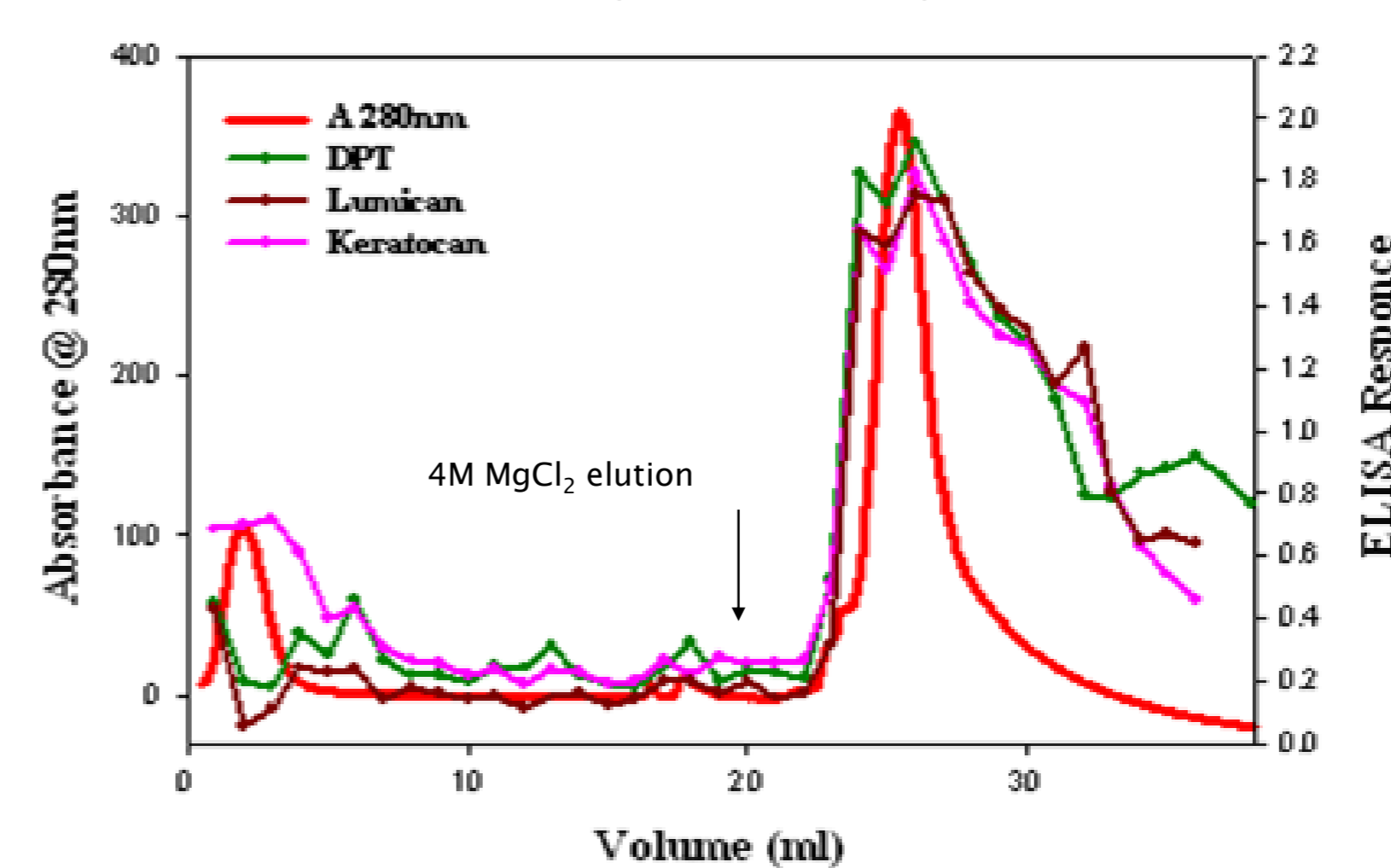
Sample in B & C were treated with β -galactosidase

Methodology



Results

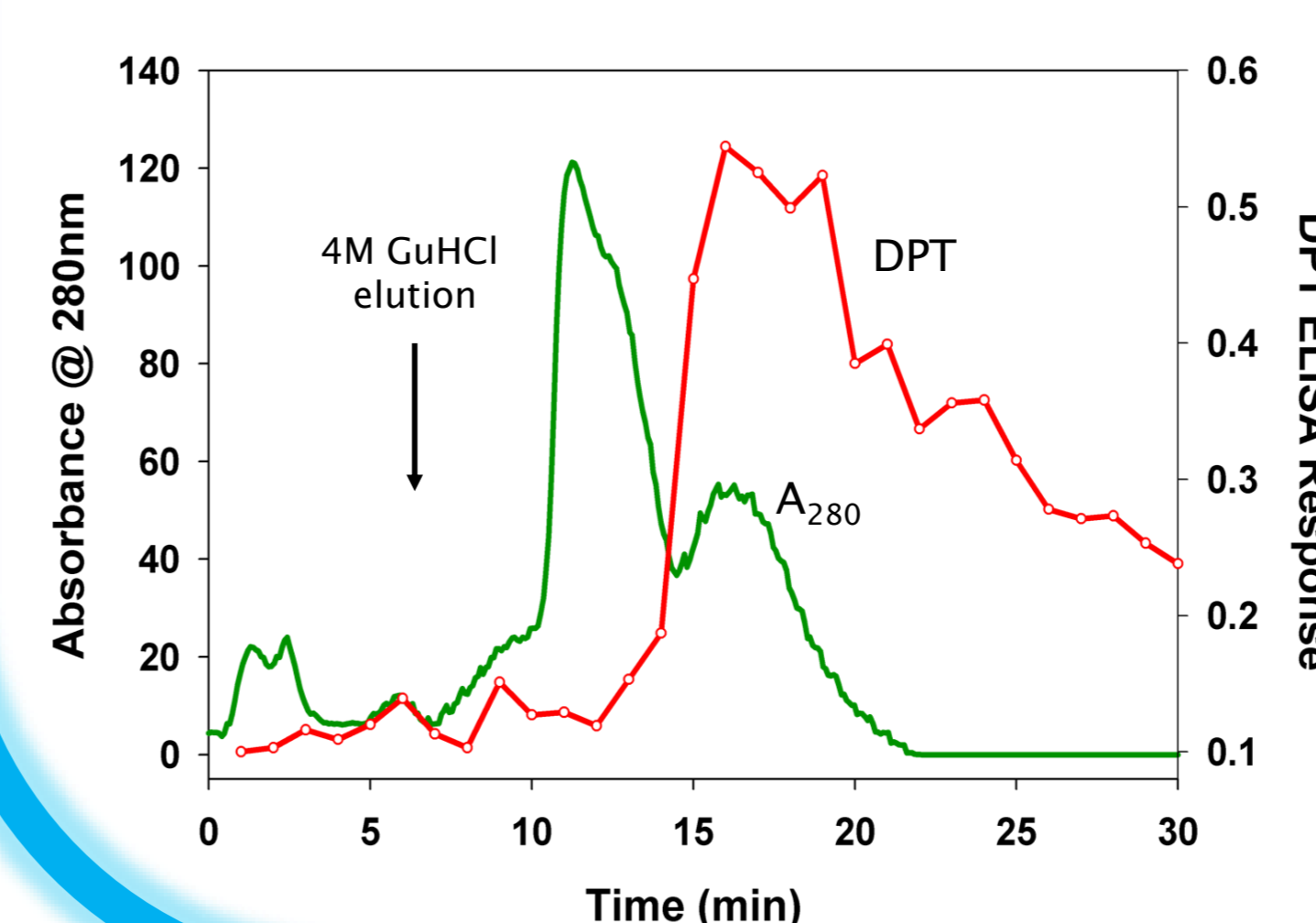
- DPT-KSPGs complex components are DPT, lumican and keratocan.



Anti-DPT Ab affinity column chromatography of the DPT-KSPGs complex.

Sample eluted with 4M MgCl₂ is positive for DPT, lumican and keratocan (ELISA response: Green is DPT, Red is lumican & pink is keratocan).

- Dermato-pontin interacts with KSPGs via their KS chains.



Anti-KS affinity column chromatography of DPT-KSPGs complex.

Sample eluted with 4M GuHCl is positive for DPT (ELISA response in red). The 280 nm response shows two population of proteins, one of which is DPT. The other contains lumican, as detected by WB (results now shown).

Conclusions

- Dermato-pontin (DPT) is essential for collagen fibrillar organisation.
- DPT interacts with the KS-PGs lumican and keratocan, via the KS chains.
- DPT may affect collagen fibril-organization via interaction with KSPGs.

References

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