Recombinant production, purification, crystallization, and structure analysis of human transforming growth factor β2 in a new conformation

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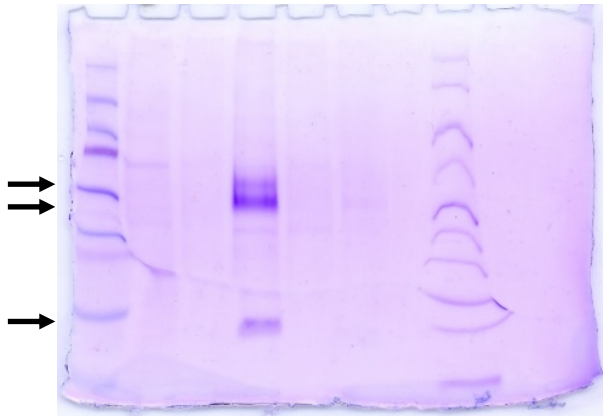
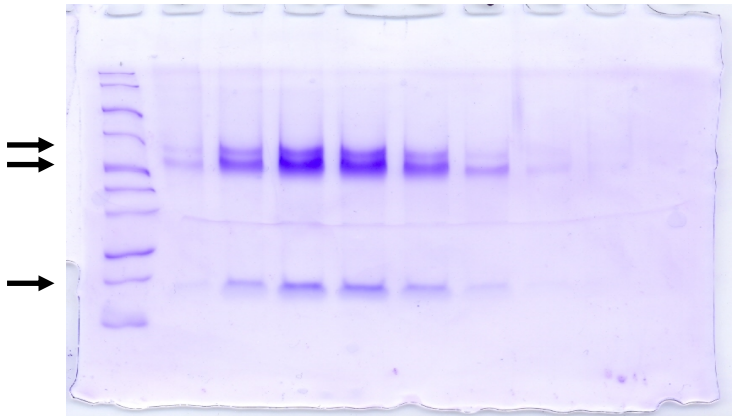
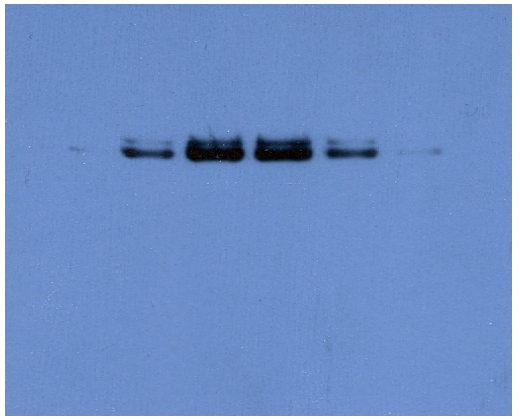
SUPPLEMENTAL FIGURE FOR REVIEW ONLY

A

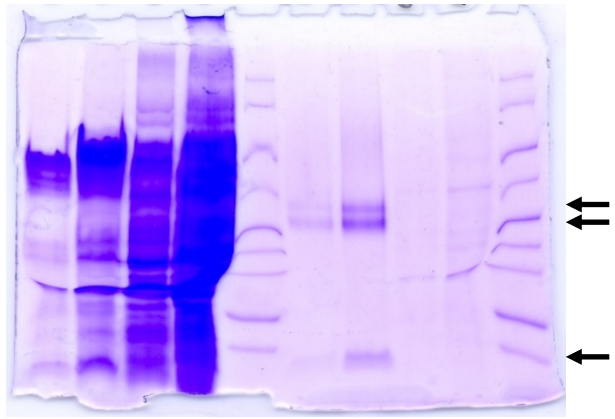

M FT W 1E 2E 3E 4E M

B

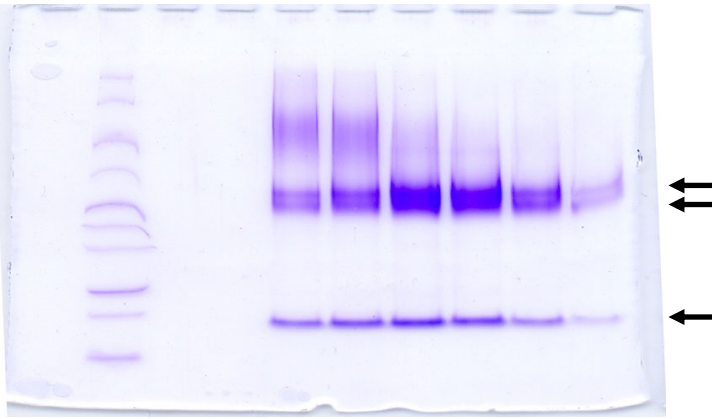

M F15 F16 F17 F18 F19 F20 F21 F22 F23

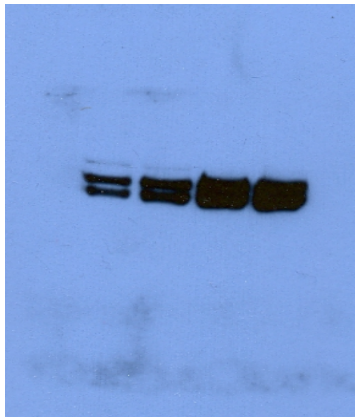

F15 F16 F17 F18 F19 F20 F21

C

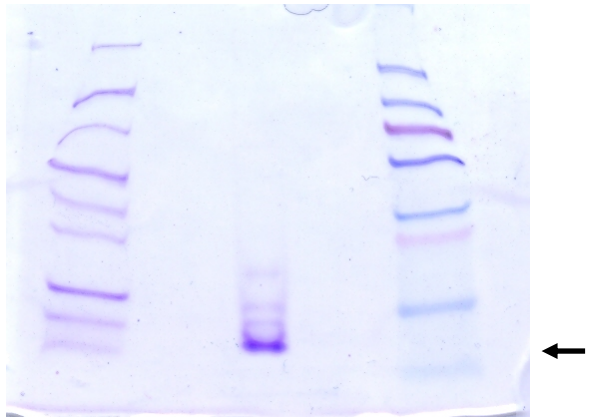

Strep-tagged protein X
Strep-tagged pro-TGFβ2
4E 3E W FT M 2E 1E W FT M

D

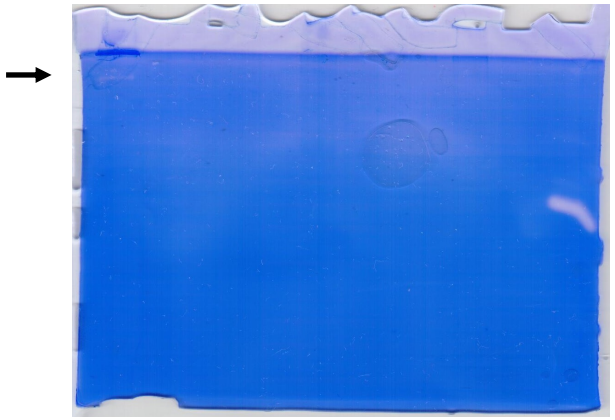

M F13 F14 F15 F16 F17 F18 F19 F20


F15 F16 F17 F18

F



G



Supplement for Figure 1 showing the entire gels of the panels depicted — Production, purification and crystallization of human TGFβ2. **(A)** Reducing SDS-PAGE depicting N-terminally octahistidine-tagged pro-TGFβ2 after Ni-NTA affinity purification. M, molecular mass marker; FT, flow-through; W, wash step; 1E, first elution; 2E, second elution; 3E, third elution; and 4E, forth elution. Black arrows pinpoint (top to bottom) intact pro-TGFβ2, LAP, and the GF in lane 1E. **(B)** Reducing SDS-PAGE of fractions (F15-F23) of the size-exclusion chromatography purification step (left panel) and Western-blot analysis of fractions F15-F21 employing an anti-histidine-tag antibody (right panel). **(C)** and **(D)**, same as (A) and (B) for N-terminally Strep-tagged pro-TGFβ2. In (C) SDS-PAGE is shared with other Strep-tagged protein. In (D), an anti-Strep-tag antibody was used. **(E)** Representative tetragonal crystals of mature TGFβ2 of ~20 microns maximal dimension. **(F)** Reducing SDS-PAGE of ~90 collected, carefully washed and dissolved diffraction-grade crystals revealing they contain mature TGFβ2 (black arrow). **(G)** Gelatin zymogram of pooled and purified crystallization drop supernatant (*leftmost lane*) showing a band pinpointed by an arrow corresponding to the mass of human α2-macroglobulin associated with gelatinolytic activity. The white band on the right corresponds to the control (trypsin).