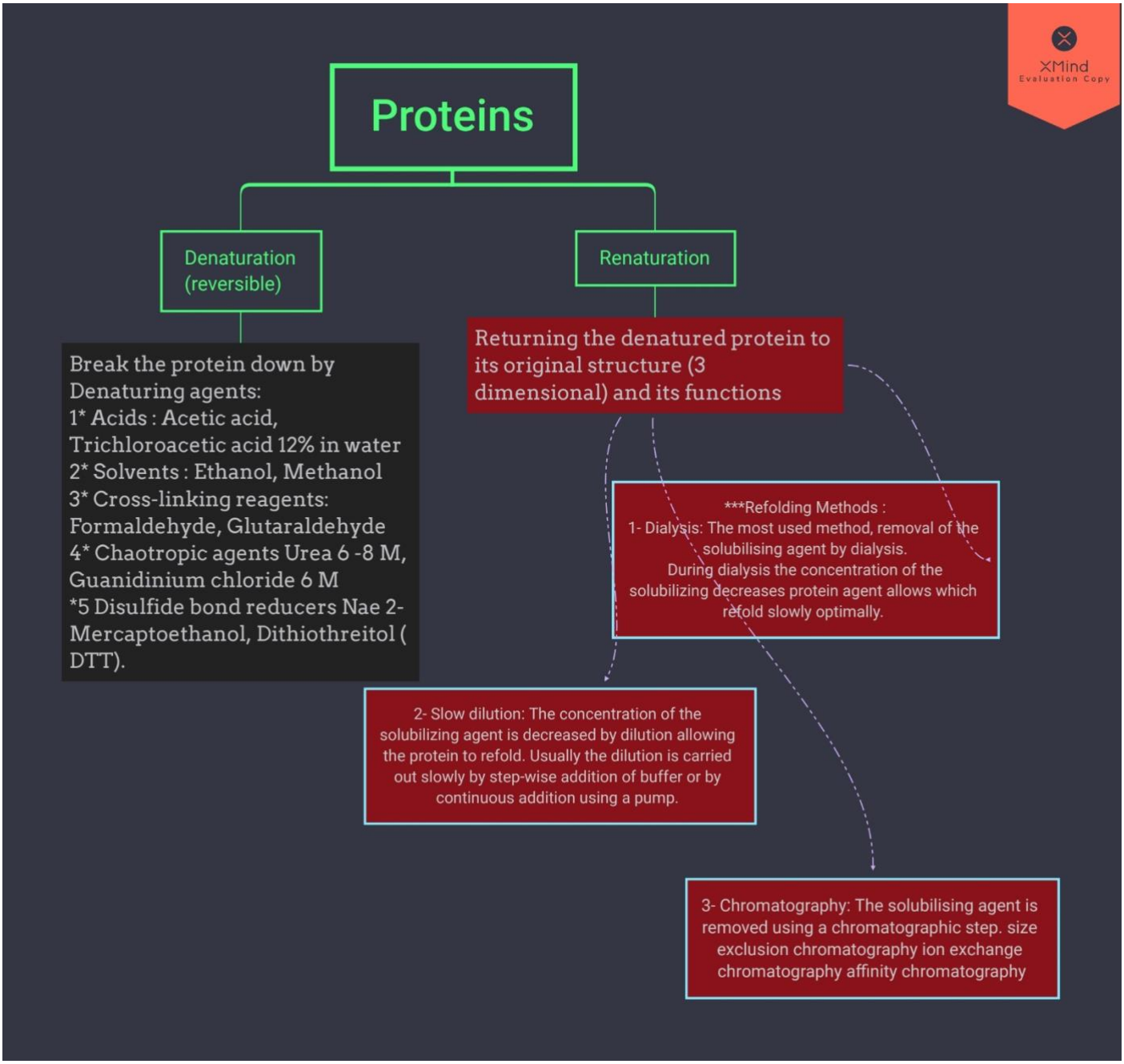
protien folding & Collagen metabolism

collagen linked diseases (slide 17)	CAUSES	COLLAGEN TYPE
EHLERS-DANLOS SYNDROME (EDS)	Mutations in the : * Fibrous proteins: COL1A1, COL1A2, COL3A1, COL5A1, COL5A2, and TNXB * Enzymes: ADAMTS2, PLOD1, B4GALT7	* I&II (classical) : inherited as autosomal dominant genetic triat * III (hypermobility) : same of I&II * IV (vaacular;the atrial form) : same of III , but recessive trait has been described * VI (Kyphoscoliosis) : autosomal recessive * VIIB (arthrochalasia) : autosomal recessive * VIIC (dermatosparaxis) : rare * Tanscin-X deficient type : autosomal recessive
COLLAGENOPATHY	* Mutations in the COL11A1, COL11A2, and COL2A1(types II and XI)	TYPE II (col2a1) XI &
ALPORT SYNDROME	* Mutation in COL4A3, COL4A4, COL4A5, Collagen biosynthesis genes * Risk Factors: 1- End-stage kidney disease in male 2- Hearing loss before age 30	TYPE IV Collagen
ULLRICH CONGENITAL MUSCULAR DYSTROPHY	* Mutation in: COL6A1, COL6A2, COL6A3 (the gene of this disease lies on Chromosome 21&2)	TYPE VI Collagen


Proteins
Denaturation (reversible)
Renaturation
Break the protein down by Denaturing agents:
1* Acids : Acetic acid, Trichloroacetic acid 12% in water
2* Solvents : Ethanol, Methanol
3* Cross-linking reagents: Formaldehyde, Glutaraldehyde
4* Chaotropic agents Urea 6 -8 M, Guanidinium chloride 6 M
*5 Disulfide bond reducers Nae 2-Mercaptoethanol, Dithiothreitol (DTT).
Returning the denatured protein to its original structure (3 dimensional) and its functions
***Refolding Methods :
1- Dialysis: The most used method, removal of the solubilising agent by dialysis.
During dialysis the concentration of the solubilizing decreases protein agent allows which refold slowly optimally.
2- Slow dilution: The concentration of the solubilizing agent is decreased by dilution allowing the protein to refold. Usually the dilution is carried out slowly by step-wise addition of buffer or by continuous addition using a pump.
3- Chromatography: The solubilising agent is removed using a chromatographic step. size exclusion chromatography ion exchange chromatography affinity chromatography
XMind
Evaluation Copy

Synthesis of Collagen

Collagen is composed of 3 polypeptide alpha chain (triple helix), identical or different.

Transcription of mRNA

collagen synthesis begins with turning on genes(34 genes associated with collagen formation) >> associated with the formation of a particular alpha peptide.

Pre-pro-peptide Formation

1- mRNA exits from the cell nucleus and enters into the cytoplasm it links with the ribosomal subunits and the process of translation occurs.

2 -The first part of the new peptide is known as the signal sequence,which is on the N-terminal of the peptide & recognized by a signal recognition particle on the ER , which will be responsible for directing the pre-pro-peptide into the ER

3 - once the synthesis of new peptide is finished, it goes directly into the ER for post-translational processing.

Alpha peptide to Pro-collagen

(1) Signal peptide on the N-terminal is dissolved (propeptide)

(2) Hydroxylation of:lysine & proline on propeptide to produce hydroxyproline and hydroxylysine

- by the enzymes prolyl & lysyl hydroxylase
- * occurs to aid crosslinking of the alpha peptides.
- *enzymatic step that requires vitamin C as a cofactor.
- * lack of hydroxylation of prolines and lysines causes a looser triple helix

(3) Glycosylation occurs by adding either glucose or galactose monomers onto the hydroxy groups that were placed onto lysines, but not on prolines.

(4) Golgi Apparatus Modification:
-In GA , the procollagen goes through one last post-translational modification before being secreted out of the cell.
-In this step oligosaccharides (not monosaccharides like step 3) are added.
then the alpha peptide is packaged into a secretory vesicle destined for the extracellular space.

(5) Formation of tropocollagen: once outside the cell, membrane bound enzymes known as collagen peptidases, remove the "loose ends" of the procollagen molecule. (tropocollagen.)

(6) Formation of the Collagen Fibril: -Lysyl oxidase and extracellular enzyme produces the final step in the collagen synthesis pathway. -This enzyme acts on lysines and hydroxylysines producing aldehyde groups, which will eventually undergo covalent bonding between tropocollagen molecules (aldol condensation reaction). This polymer is known as a collagen fibril.