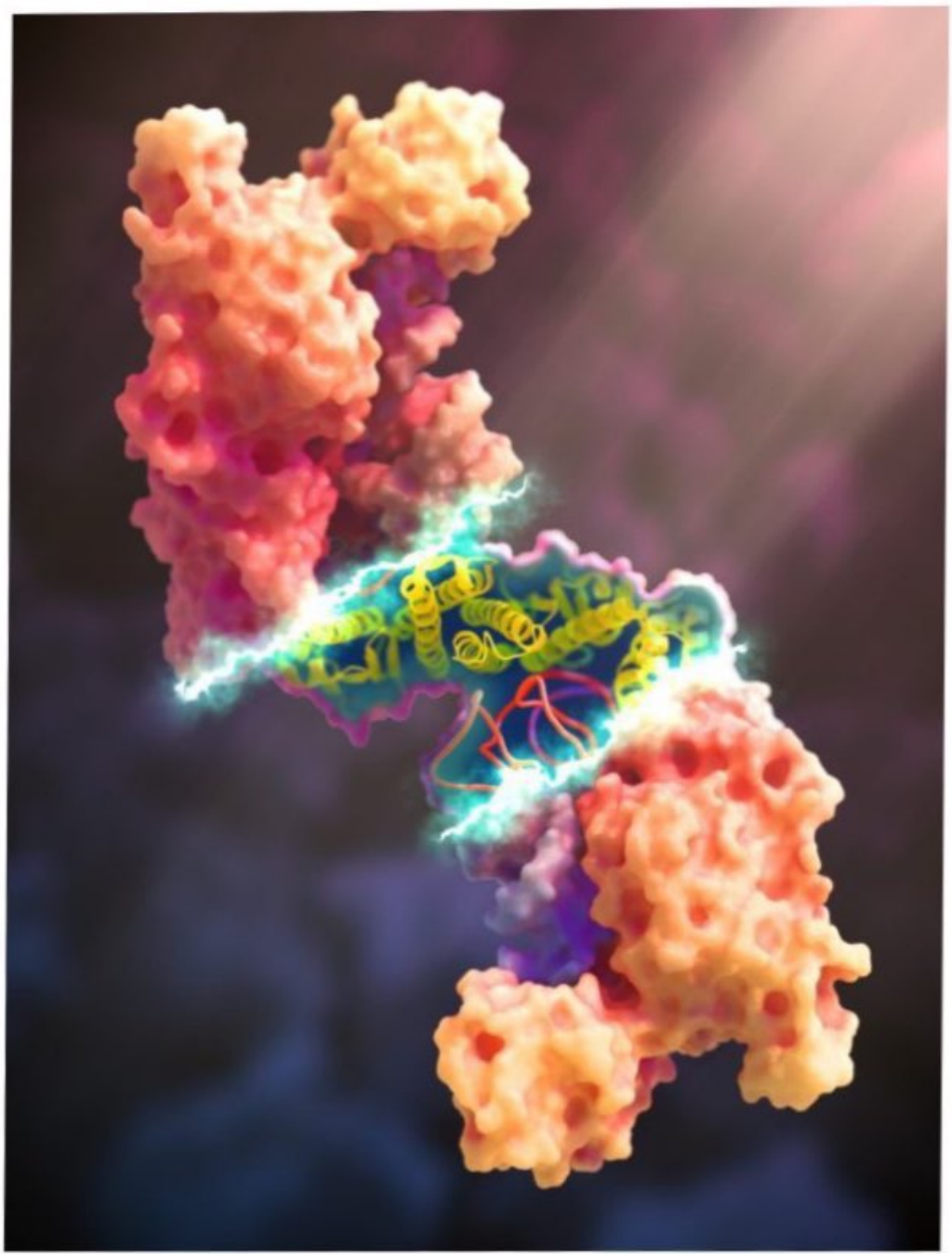
* Quantitation of protein .. ♡



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📌عملية تحديد كمية البروتين مُهمة جدًا ومطلوبة قبل ما اقبل اعمل processing للبروتين لل isolation, separation, analysis..
عن طريق إما:

Immunochemical, electrophoretic, chromatography method.

📌كيف بدي أختار ال method المناسبة !
هاد الشي بعتمد على مدى توافق العينة مع ال method بحيث تعطيني
Less pre-treatment & less manipulation ..

📌أكبر مشكلة وتحدّي ممكن يواجه الباحثين هو ال standard ..
ممكن إنه ما يتفاعل بشكل جيّد مع ال reagent بالتالي بيعطيني خطأ في ال protein essay ..

📌في عندي تحديات أُخرى ممكن تواجهني متل ال purity للبروتين + الكلفة اله لأن ممكن يكون غالي !!

📌ممكن ما يتوفر عندي ال highly purified version of the protein أو ال cost اله كتير عالي ومُكلف ممكن أستخدم واحد آخر بس بشرط !
يعطيني similar color + نفس ال protein essay method .. response curve with the selected

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- The most two common colorimetric methods:
  - Protein assay reagents involve either protein-dye binding (e.g coomassie) chemistry
  - Protein-copper chelation chemistry.

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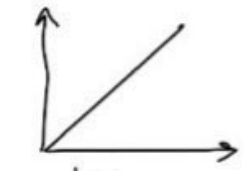
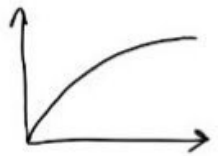
* Advantages: (BSA) (1) work well for a protein Standard.

* 1. High purity
* 2. relatively inexpensive.

(2)
* BGG → For a Mixture Containing Several immunoglobulins.
  → Determine the Conc. of antibiodies.
  ↓
  produce a color response Curve very Similar to → IgG

العلاقة بين الconc. وال Abs





بعدها بعمل estimations ل
total protein
concentration للعينة اللي

Linear

* fewer point (2-3)

Non-Linear

* بحاجة لعينات أكثر..
* at Least (2) protein..

بكرر الـ test مرتين لكل Conc.

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قبل ما أعمل analysis للبروتين في عدة أشيا لازم أعملها ..

📌 stabilization عن طريق إضافة buffer

📌 inhibition لل microbial growth عن طريق إضافة antibiotic لأن البروتين عبارة عن وجبة للبكتريا..

📌 أتجنب ال contamination with other foreign substance متل ال hair, dust, skin, body oils عن طريق ال centrifugation..

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ممكن يكون عندي non protein substance

1. ممكن افصل البروتين لحال بال gel filtration ..
2. ممكن اعملها precipitation بال centrifugation..
3. إذا كان التخلص منها صعب بعمل dialysis

لما يكون عندي blood sample وبدي اقيس البروتين اللي فيه !

1. باخد عينة الدم وبضيف anticoagulant عشان الclotting factors ما تروح من العينة ونحصل بلازما
2. بعملها centrifugation ويحصل البلازما اللي بتحتوي على كل البروتينات بالدم

لما تكون العينة عبارة عن tissue او cell طريقة الحصول على البروتينات مختلفة بعمل solubilization processes :

1. بضيف surfactant عشان اضعف ال cell wall واكسره
2. اعمل grinding طحن للانسجة
3. اخر خيار ال sonication لتكسير الخلايا.

📌 البروتين ممكن تكون بال cytosol او بال membrane ..

📌إذا بشتغل على خلايا بكتيرية أو خلايا إنسان لازم اضيف ال protease inhibitors عشان لما اكسر
📌الخلايا ال proteas رح تبلش تطلع وممكن انها تخرب ال protein ..
الباقي للخلايا ممكن اشيلها عن طريق ال filtration او centrifugation ..

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METHODS TO DETERMINE PROTEIN CONCENTRATION

- The criteria for choice of a protein assay are usually based on:
  - Convenience
  - Availability of protein for assay
  - Presence or absence of interfering agents,
  - Need for accuracy.

- The Lowry method is very sensitive but is a two-step procedure that requires a minimum of 40 minutes incubation time. The Bradford assay is more sensitive and can be read within 5 minutes, however proteins with low arginine content will be underestimated.
- Generally, estimates are more accurate for complex mixtures of proteins. Estimates of concentration of pure proteins can be very inaccurate depending on the principle of the assay, unless the same pure protein is

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METHODS TO DETERMINE PROTEIN CONCENTRATION

- For this experiment you will determine the protein concentration of your unknowns using a calibration curve from a standard solution of bovine serum albumin (BSA) using both the Biuret and Bradford methods.
- To do this you will start by constructing a standard curve (absorbance vs. amount of protein) using a standard solution of BSA (1.0 mg/mL) for each assay

Table 1

Method	Sensitivity range	Volume of sample needed	accuracy	convenience	Interfering agents
Absorbance at 280 nm	20µg-3mg	200µl-3ml	fair	Excellent when equipment's available	Detergents, nucleic acids, particulates, lipid droplets
Modified Lowry	2-100 µg حساسة	1 ml	Good	Fair	Strong acids, ammonium sulphate
Biuret	1-10 mg not good	5 ml	Good	Good	Ammonium salts
Bradford	1-20 µg (micro) 20-200µg (macro)	1ml (micro) 5.5ml (macro)	Good	Excellent	None

أكثر استخدام

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9) Biuret Method

* is similar to that of Lowry.

الاختلاف:

* incubation period: ← ال Biuret أقل بكثير 20
* interfering Substances: ← ال Biuret أقل بكثير

* السلبيات إلها..

(1) بستهلك مواد وكيموات كثيرة .

(2) ال Sensativity قليلة بالمرة .

استخداماتها:

(1) كميات كبيرة من المادة .

* ال Batches

which yield is not problem

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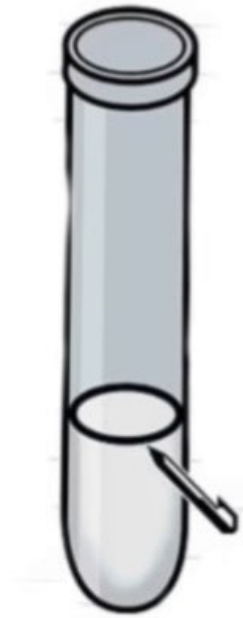

Biuret Method procedure .. ♡♡

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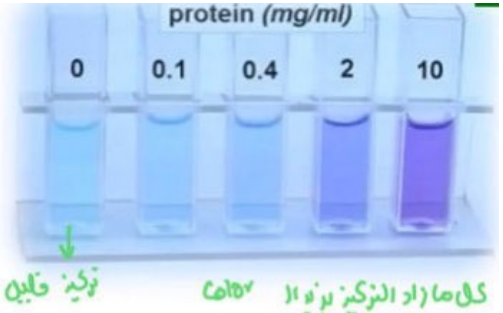
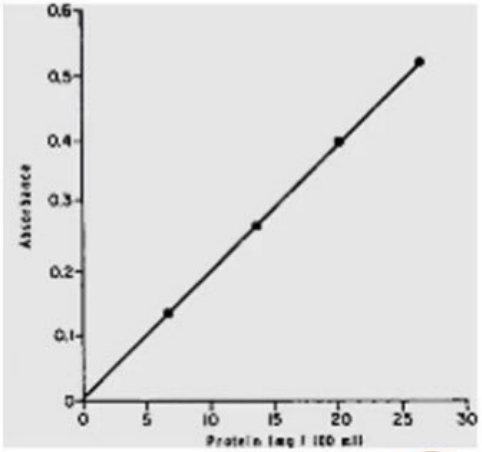
BIURET METHOD PROCEDURE:

- Dilute samples to concentrations 0, 1, 2, 4, 6, 8 and 10 mg/ml with buffer. Add 0.1 ml to each assay tube. Duplicate samples are recommended, and a range of dilutions should be used.
- Prepare a reference tube with 1 ml buffer.
- Prepare standards from 10 mg/ml bovine serum albumin. Range should be from 1 to 10 mg protein.
- Add 0.9 ml Biuret reagent to each tube, vortex immediately, and let to stand for 20 min.
- Read at 550 nm.
- Prepare a standard curve of absorbance versus micrograms protein (or *vice versa*), and determine mounts from the curve. Determine concentrations of original samples from the amount protein, volume/sample, and dilution factor, if any.





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📌 ال color لل biuret method بتكون ثابتة .. ومع ذلك لازم اقرأ كل القراءات، ومجرد خلصت ال١٠ د مو لازم استنى لحتى اقرأ كل القراءات ..

📌 ال cuvette اللي عندي حجمها مو لازم يكون كبير يعني تقريبًا 4 mL عشان أوّفر بال reagent وال protein sample اللي عندي فممكن اعملها scaling down بدل 4 mL بخليها 1 mL ..

📌 ال proteins اللي فيها aromatic amino acid متل ال tryptophan, phenylalanine, tyrosine, Histidinee ممكن يعطوني قراءة اعلى لل Abs لما يتفاعل مع ال copper..

📌 اللي عندهم Low بال amino اللي عندهم aromatic ممكن يعطيني قراءة أقل !

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Bradford Method

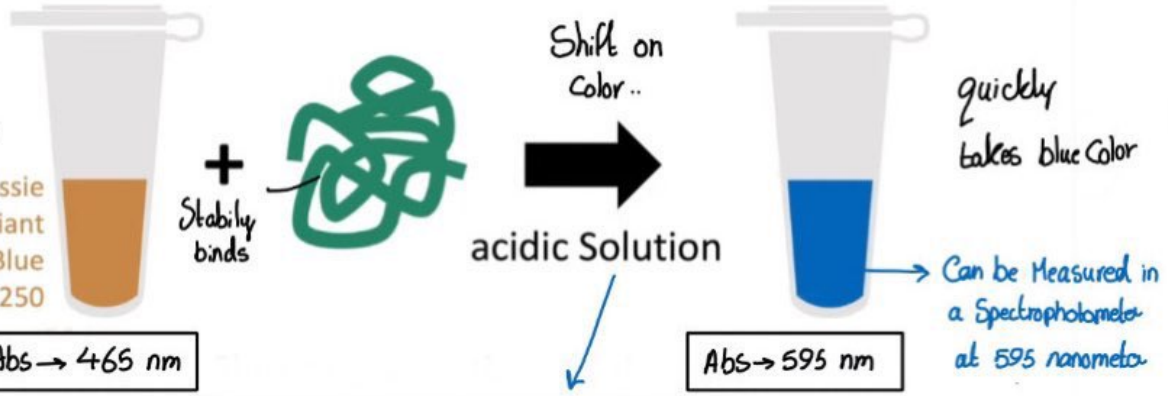
→ More Sensitive Method (20-200 μg)

* used to determine the Conc. of protein in a Solution.

* طريقة بستخدمها عشان أحدد تركيز البروتين بالمحلول..

based on:

dye: reagent. Coomassie Brilliant Blue G-250



prolonated and has reddish or brownish Color.. ←

Abs → 465 nm

quickly takes blue Color

→ Can be Measured in a Spectrophotometer at 595 nanometer

Abs → 595 nm

required for this reaction is an acidic environment

* الصبغة ممكن تعاني Colorization ال Cuvettes

* الأفضل يكون استخدم ال Disposable Polystyrene Cuvettes

* in the absence of protein there is no change in color

* اللي بيعطي Binding ال Stabilization :

1. Hydrophobic
2. ionic interaction

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BRADFORD METHOD PRINCIPLE

- **Bradford reagent** Dissolve 100 mg Coomassie Brilliant Blue G-250 in 50 ml 95% ethanol, add 100 ml 85% (w/v) phosphoric acid. Dilute to 1 liter when the dye has completely dissolved, and filter through Whatman #1 paper just before use.
- 1 M NaOH can to be used if samples are not readily soluble in the color reagent.
- The Bradford reagent should be a light brown in color. Filtration may have to be repeated to rid the reagent of blue components. The Bio-Rad concentrate is expensive, but the lots of dye used have apparently been screened for maximum effectiveness.

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مهم أعرف ليه بضيف ال NaOH (أحيانًا) عشان يزيد ال solubility اله ..
في حال صار مشكلة وما قدر يصير soluble بال color reagent ..

بزيد الsolubility ال
membrane البروتين بالتالي
بعطيني قراءة أدق وأصح..

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BRADFORD METHOD ASSAY

- Dilute samples with buffer to an estimated concentration of 20 to 200 micrograms/ml
- Prepare standards containing a range of 20 to 200 micrograms protein (albumin or gamma globulin are recommended) to a standard volume (generally 1 ml or less).
- Prepare unknowns to estimated amounts of 20 to 200 micrograms protein per tube, same volume as the standards.
- (optional) Add 0.25 ml 1 M NaOH to each sample and vortex.
- Add 5 ml dye reagent and incubate 5 min.
- Measure the absorbance at 590 nm.

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Advantages / Disadvantages for Bradford test.

Advantages

1. Quickly or very fast..

   Easy to use since it just requires the bradford reagent.

2. Extremely Sensitive..

   (it Can even detect protein Conc. of one Microgram permilliter..

Not Accurate

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ال reagent اللي عندي بتفاعل مع ال arginine أكتر شي، وبدرجة اقل مع ال aromatic ..
فال accuracy لل acidic وال basic ممكن تختلف وتصير قليلة ..

بالنسبة لل standard protein ممكن استخدم : BSA, IgG

بستخدمها في حال كانت البروتينات Gamma globulin
نوعها Globulin..

نوع مختلف ممكن استخدم ال BSA ..

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ANALYSIS

- Prepare a standard curve of micrograms protein versus absorbance, and determine amounts from the curve. Determine concentrations of original samples from the amount protein, volume/sample, and dilution factor, if any.
- The dye reagent reacts primarily with **arginine** residues and less so with histidine, lysine, tyrosine, tryptophan, and phenylalanine residues.
- The assay is less accurate for basic or acidic proteins. The Bradford assay is rather sensitive to **bovine serum albumin**, more so than "average" proteins, by about a factor of two. **Immunoglobulin G** (IgG - gamma globulin) is the preferred protein standard.
- The addition of 1 M NaOH allows the **solubilization of membrane proteins** and reduce the protein-to-protein variation in color yield.

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- Prepare the following dilutions:

Tube	BioRad Dye (uL)	Buffer (uL)	Vol Standard (uL)
1	1000	20	0
2	1000	18	2
3	1000	16	4
4	1000	14	6
5	1000	12	8
6	1000	10	10

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