Backreflection

Backreflection is a biophysical characterization technique used for assessing the colloidal stability of a biological sample.

It is a highly sensitive technique that uses the intrinsic light scattering attributes of a sample to measure its light attenuation and monitor the turbidity of that sample.

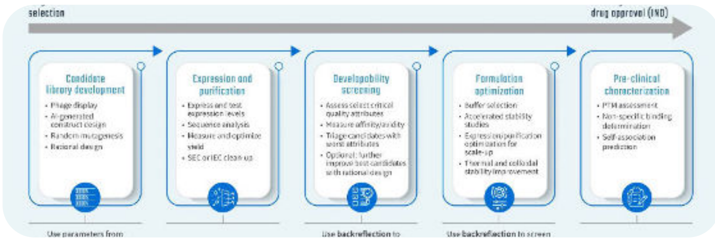
Backreflection is a valuable tool for many researchers in therapeutic spaces

Backreflection is a useful tool for anyone developing biologics or gene therapies. Complex biological samples such as enzymes, monoclonal antibodies, or AAVs that will be used for treatments must be highly stable for storage, transport, and clinical administration.

Backreflection enables you to monitor the turbidity of your sample. This information helps you optimize your sample by making changes to the sequence or buffer environment, and measuring how those changes impact stability. Stability parameters are used to rank candidates for therapies and select those with greater stability for further characterization and development.

When used in parallel with other biophysical techniques, backreflection tells you about the overall stability of a sample. Use it for:

- Pre-formulation phase
- Developability assessment
- Buffer formulation and optimization



Backreflection provides insight about the turbidity of your samples

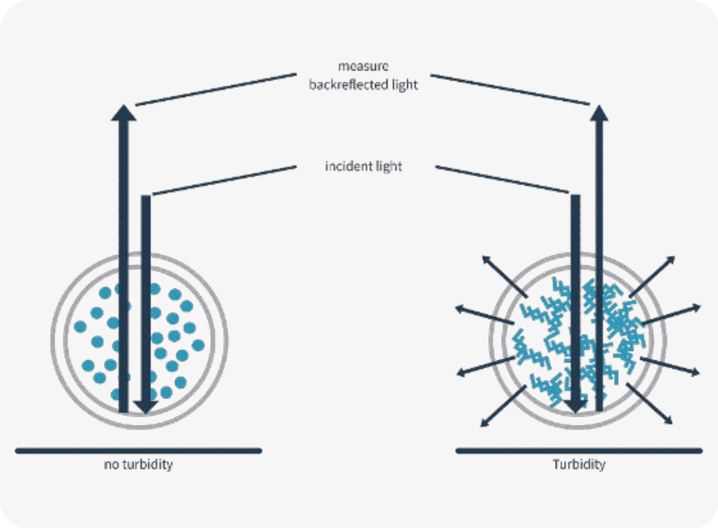
Turbidity is the “cloudiness” of your sample – that is, how well light passes through it. If a protein sample is highly turbid, it’s an indicator that there is aggregation or some other large particle contamination.

Changes to the primary sequence or buffer environment impact the stability of a protein. When screening libraries of candidates or conditions, it’s important to only pass on candidates with improved stability. Turbidity data from backreflection measurements enable ranking candidates for increased stability attributes.

Light scattering information is also used to assess colloidal stability; however, backreflection is more sensitive for certain sample types. Further, the interplay between protein unfolding and the formation of amorphous aggregation is easier to determine using both methods in parallel.

Onset of turbidity

Rr T_{turb}, is the temperature along a thermal denaturation gradient at which the sample begins to exhibit sights of turbidity. This is generally an indicator of instability and aggregation of the sample.



Large, amorphous protein aggregates scatter more light away from the sample volume, thereby reducing or attenuating the light that returns to the detection optics.

Tightly folded proteins that are highly stable do not deflect much light. As aggregates form, whether from prolonged exposure to solution or from stress inputs such as temperature or chaotropes, they clump up. As these aggregates form, they grow large compared to the folded protein, and start to deflect more light out of the sample.

The input light reflects off a mirror and returns into the system. If a substantial amount of light is deflected, it is an indicator that the sample is turbid. It is possible to monitor the increase in the turbidity signal as a way of monitoring the formation of aggregation.

Highly sensitive optics enable you to monitor the turbidity change attributed to aggregation due to either thermal or chemical denaturation.