CYCLOLAB

The Cyclodextrin Company

Cyclodextrin – protein interactions

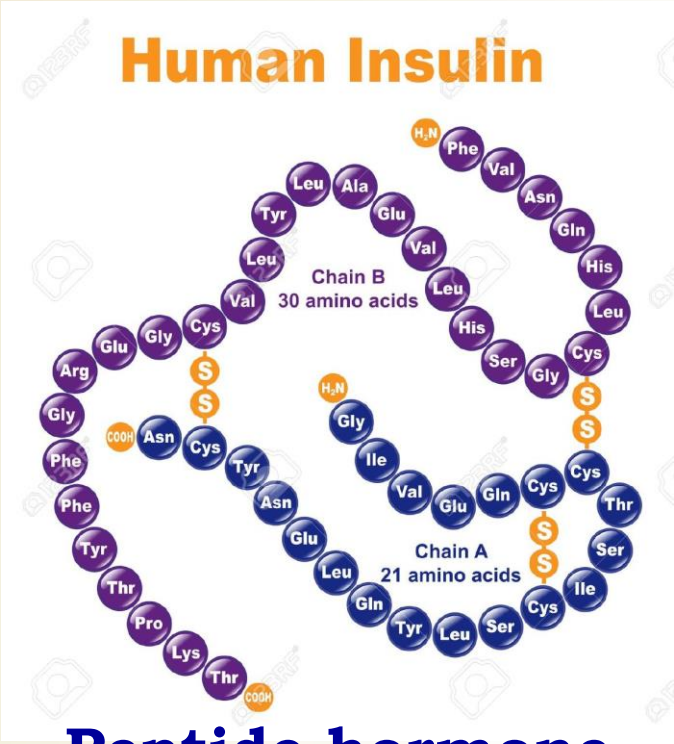
Practical aspects

Cyclodextrin – protein interactions

Cyclodextrins are molecular containers. As of 2017, 61 products of small molecules are formulated with CDs.

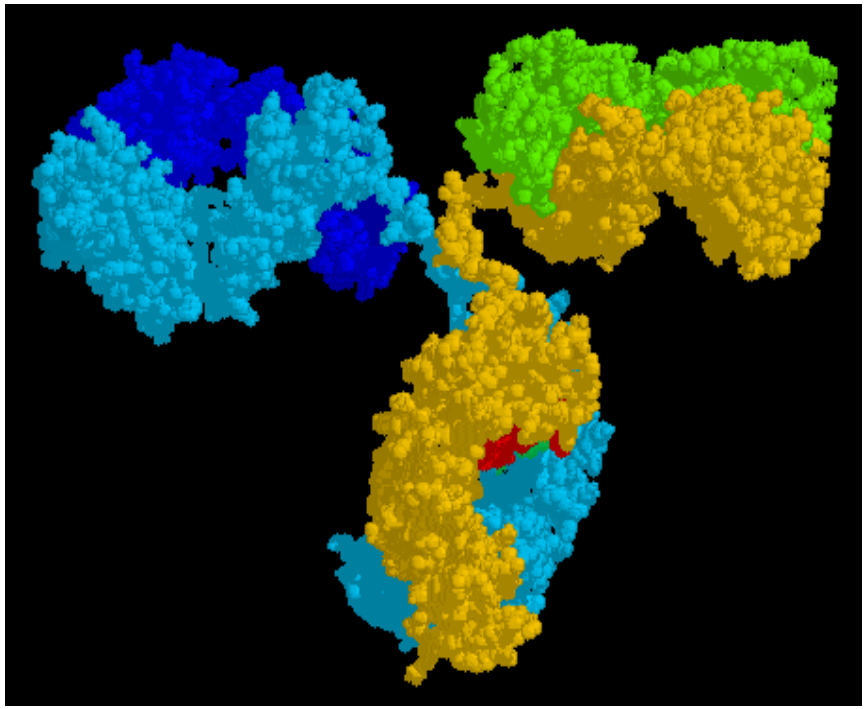
Cyclodextrin – protein interactions

Biological active substances (big molecules), proteins, peptides, monoclonal antibodies gained immerse interest in product development recently



Peptide hormone

5808 Da



Monoclonal antibodies

~1300 amino acids, 150 000 Da

Cyclodextrin – protein interactions

Why to use CDs in protein and biological formulations:

- Safer than most current excipients (e.g. Tween) – no peroxide formation, corresponding immunogenicity, degradation
- Prevention of aggregation, delay folding
- Less protein adsorption onto container surface
- Reduce/maintain viscosity
- Improve injectability
- Physical and chemical stabilization of proteins
- Life-cycle management

Cyclodextrin – protein interactions

Cyclolab has recently been putting immerse efforts to develop cyclodextrins that are particularly suitable for excipients in protein formulations.

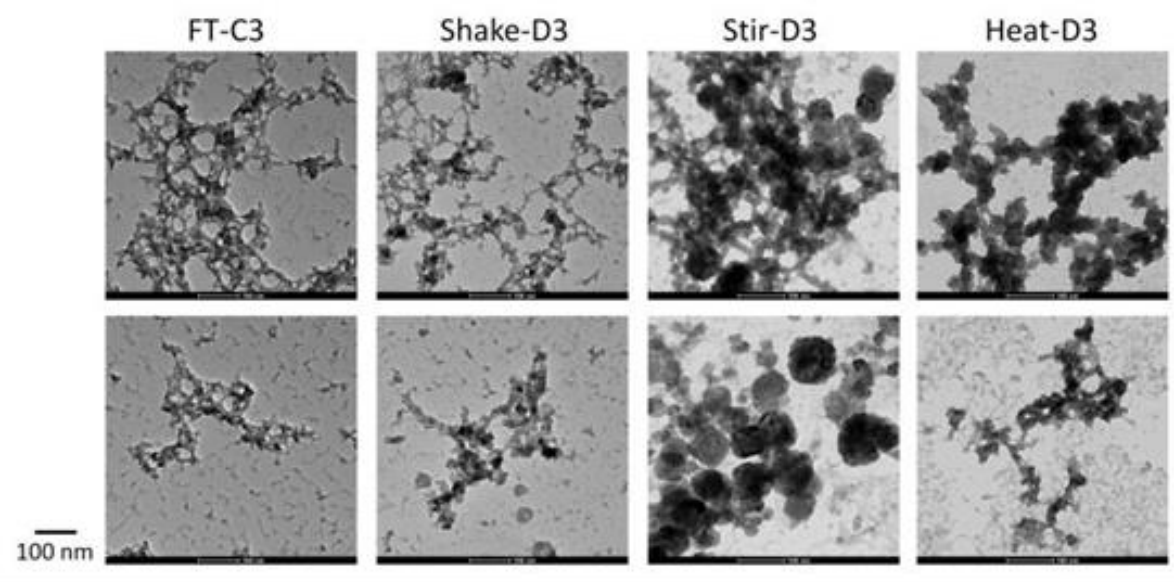
During these studies, several therapeutically relevant model compounds are included, yet we are open to test how well our CDs fit your particular monoclonal antibody or other type of protein.

Cyclodextrin – protein interactions

Outcomes of protein aggregation

- **Decreased efficiency**
- **Altered pharmacokinetics**
- **Immunogenicity, irritation, anaphylaxis**
- **Short shelf-life, poor stability**

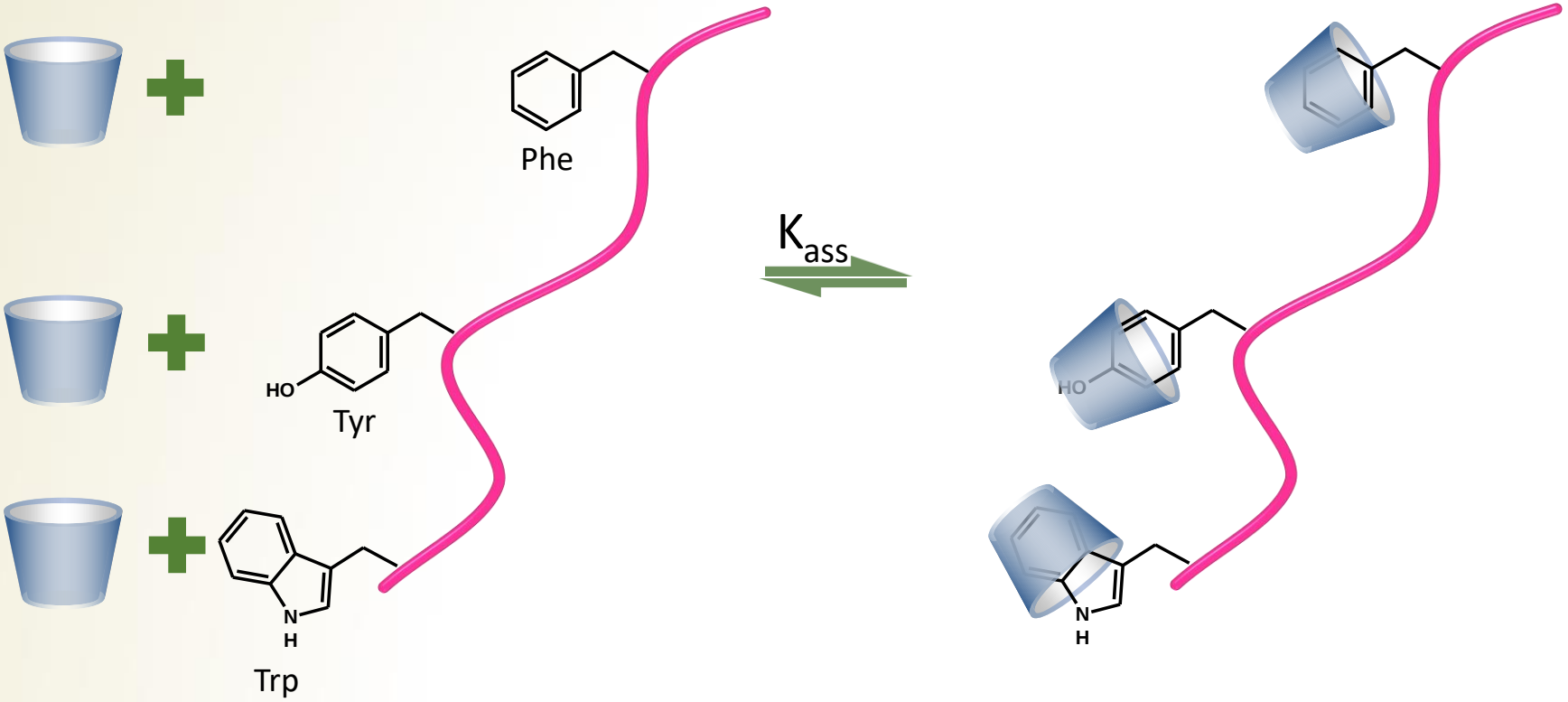
mABs are particularly prone to aggregation



Hartl (2013)

Cyclodextrin – protein interactions

Cyclodextrins are able to interact with proteins and polypeptides on several levels. The classical inclusion involves aromatic amino acids



J Szejtli, L Szente (1980)

Cyclodextrin – protein interactions

Main characteristics of the CD-protein interactions are:

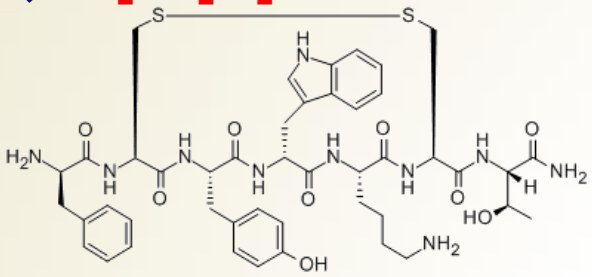
- CDs attract to hydrophobic regions of the proteins
- The interactions are host-guest and electrostatic type
- CDs act like artificial chaperons
- Certain CDs act like chaotropic agents and delay protein-protein interaction and thereby folding in solution

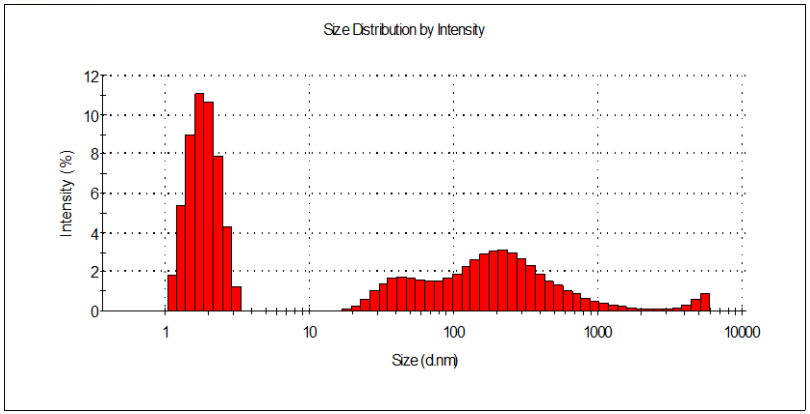
Han (2007), Serno (2010)

Cyclodextrin – protein interactions

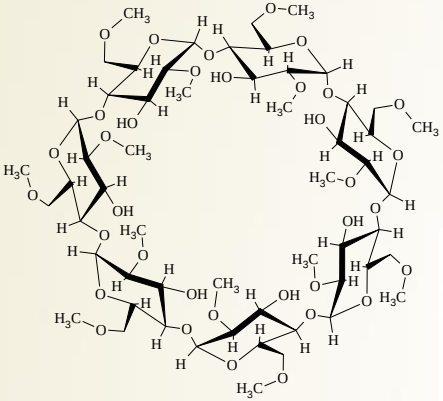
Cyclodextrin's effect on peptide aggregation

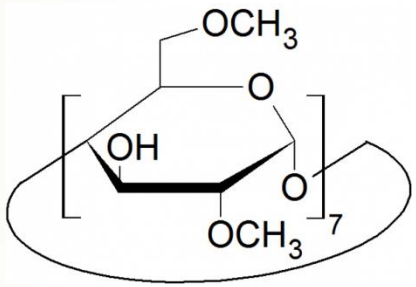
TT-232, heptapeptide

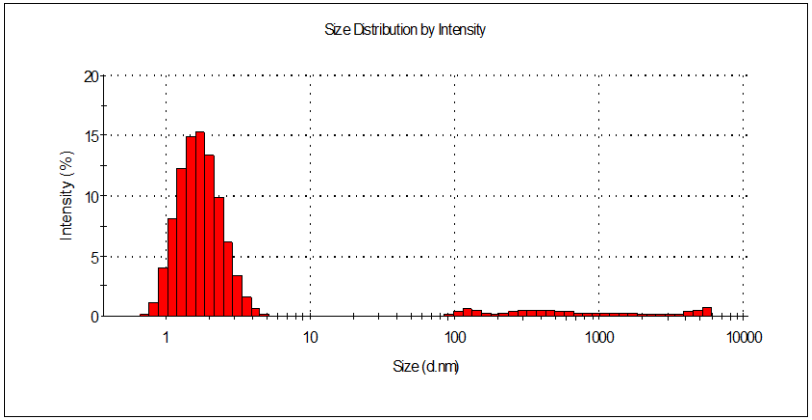




Without additive





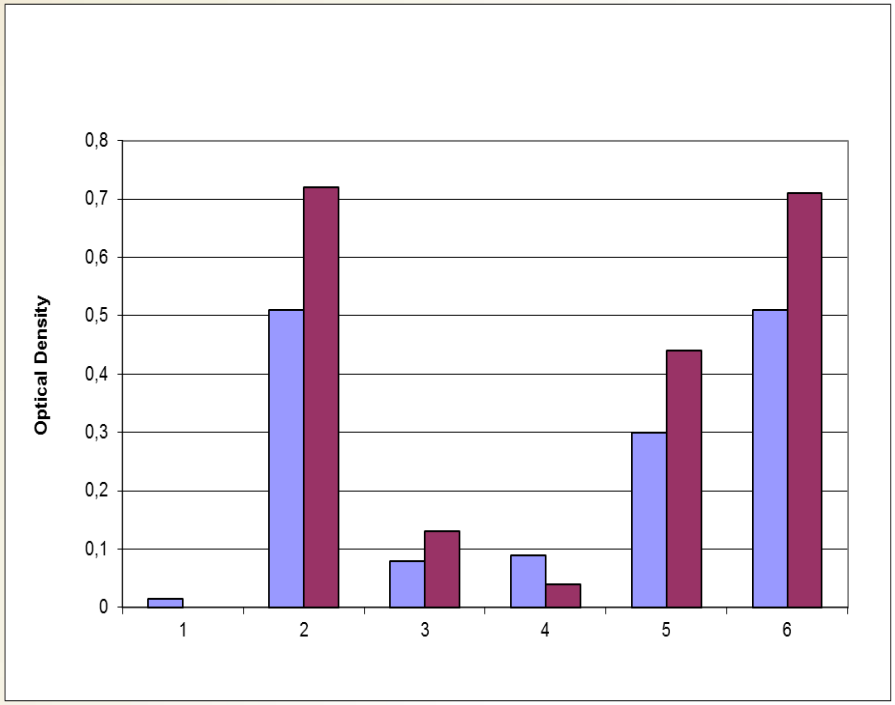


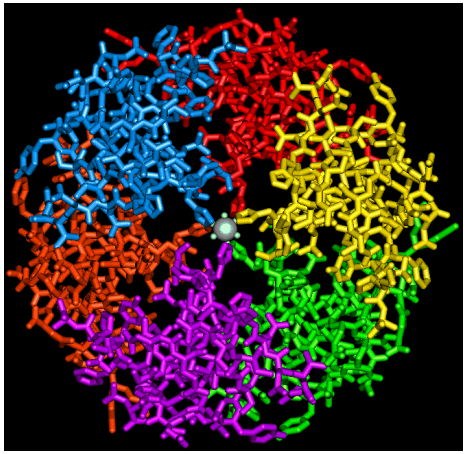
With DIMEB-CD

Kéri (2007)

Cyclodextrin – protein interactions

Cyclodextrin's effect on insulin aggregation





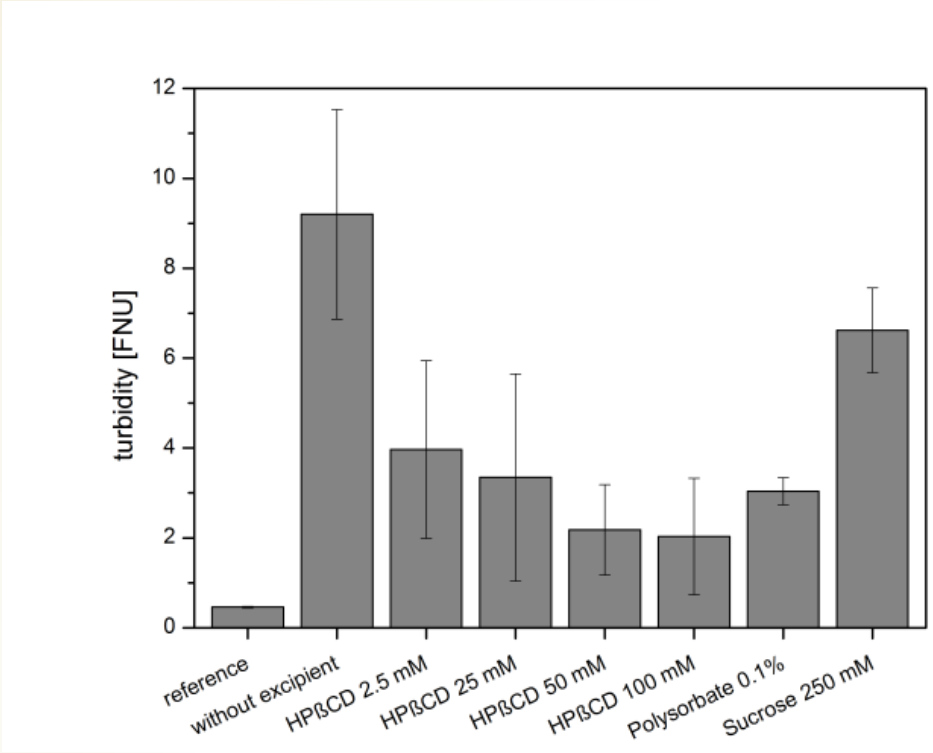
insulin hexamer

1. Control, 2. Stirred without CD, 3. HPBCD,
4. alfa-CD, 5. beta-CD, 6. gamma-CD

Banga (1993)

Cyclodextrin – protein interactions

Cyclodextrin's effect on Ig B aggregation



Turbidity of 1.8 mg/mL IgGB aqueous solution after 1 h stirring

Hartl (2013)

Cyclodextrin – protein interactions

Cyclodextrins or TWEEN?

- **Tween-like detergents efficiently prevent protein aggregation, prevent immunogenicity**
- **Polysorbate 80 is the most common, they are widely used**
- **However, their degradataion leads to aldehydes, epoxy-acids and peroxides damaging proteins**

Kishore (2011)

Cyclodextrin – protein interactions

Some protein based products, their shelf-lives and additives

Product	Active	Shelf Life	Ingredients
Remicade	infliximab	3 years at 2 °C – 8 °C.	dibasic sodium phosphate dihydrate, monobasic sodium phosphate monohydrate, **polysorbate 80**, and sucrose. No preservatives are present.
Humira	Adalimumab	2 years at 2 °C – 8 °C.	sodium chloride, monobasic sodium phosphate dihydrate, dibasic sodium phosphate dihydrate, sodium citrate, citric acid monohydrate, mannitol, **polysorbate 80** and water for injections
Herceptin	trastuzumab	4 years at 2 °C – 8 °C.	histidine hydrochloride, histidine, trehalose dihydrate, **polysorbate 20**
Vetsulin	porcine insulin zinc suspension	**42 days**	zinc chloride, methylparaben, sodium chloride, sodium acetate, water
NovoLog	insulin aspart	**28 days**	glycerin, phenol, metacresol, zinc, disodium hydrogen phosphate dihydrate, sodium chloride and water for injection
ORTHOCLONE	muromonab-CD3	**9 months**	sodium phosphate, monobasic sodium phosphate, dibasic sodium chloride **polysorbate 80** water
Oncaspar	pegaspargase	**8 months (2-5°C)**	dibasic sodium phosphate dihydrate, dibasic sodium phosphate heptahydrate, sodium chloride, water for injection

Cyclodextrin – protein interactions

Cyclodextrins and mAbs

mAb: polyionic protein with hydrophobic surfaces

- **The hydrophobic surface induces aggregation**
- **The net surface charge is minimal at pH 5 - 7.5 → no electrostatic repulsion**

Consequence: mAb-mAb aggregation increases

- **Cyclodextrin masking of the hydrophobic surface reduces van der Waals interactions**
- **Increase in the surface charge increases electrostatic repulsion**

Result: mAb-mAb aggregation decreases

Cyclodextrin – protein interactions

Company contacts:

CycloLab Cyclodextrin Research & Development Laboratory Ltd.

Budapest, P.O. Box 435, H-1525 Hungary

Location: Illatos út 7., Budapest, H-1097 Hungary

TEL: (+36) 1-347-60-60 or -70; FAX: (+36) 1-347-60-68

E-mail: info@cyclolab.hu; Homepage: http://cyclolab.hu/

Contact person: Tamas Sohajda

R&D Director

info@cyclolab.hu **Tel: (+36) 1-347-60-72**