CYCLOLAB

The Cyclodextrin Company

# Cyclodextrin enabled biologics

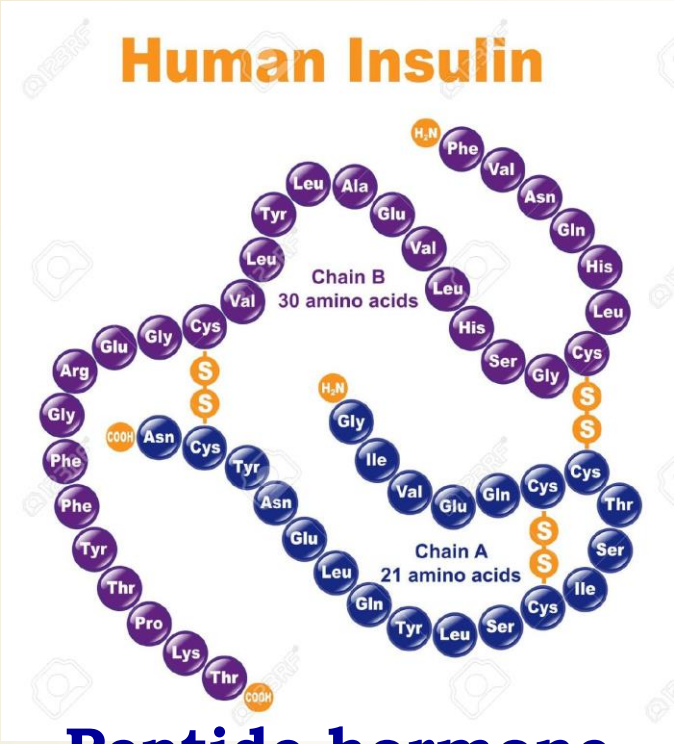
## A novel way of utilizing CDs

# Cyclodextrin – protein interactions

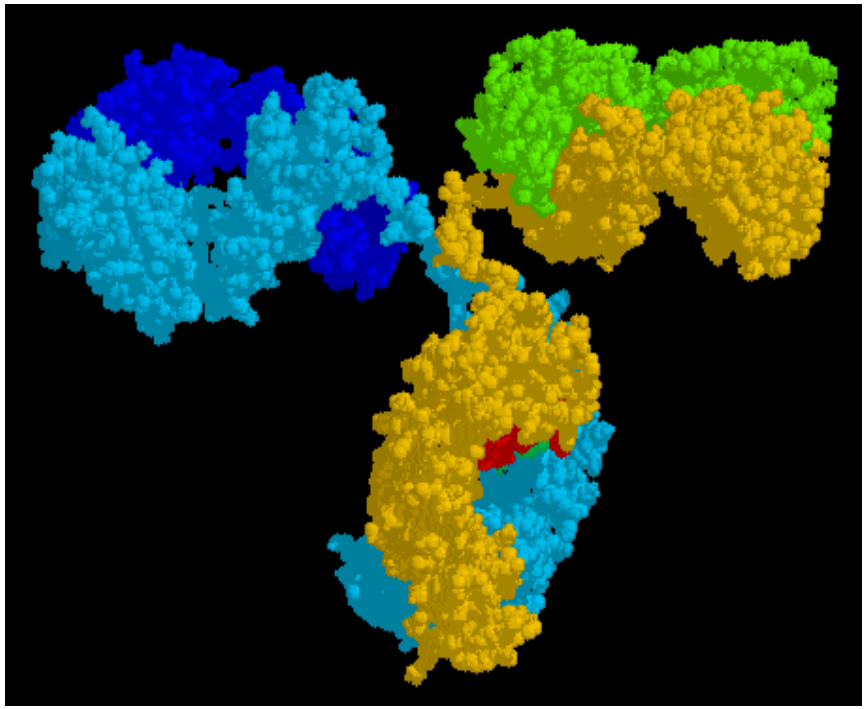
**Cyclodextrins are molecular containers. As of 2017, 61 products of small molecules are formulated with CDs.**

# Cyclodextrin – protein interactions

**Biological active substances (big molecules), proteins, peptides, monoclonal antibodies gained immense 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 immense efforts to develop cyclodextrins that are particularly suitable for excipients in protein formulations.**

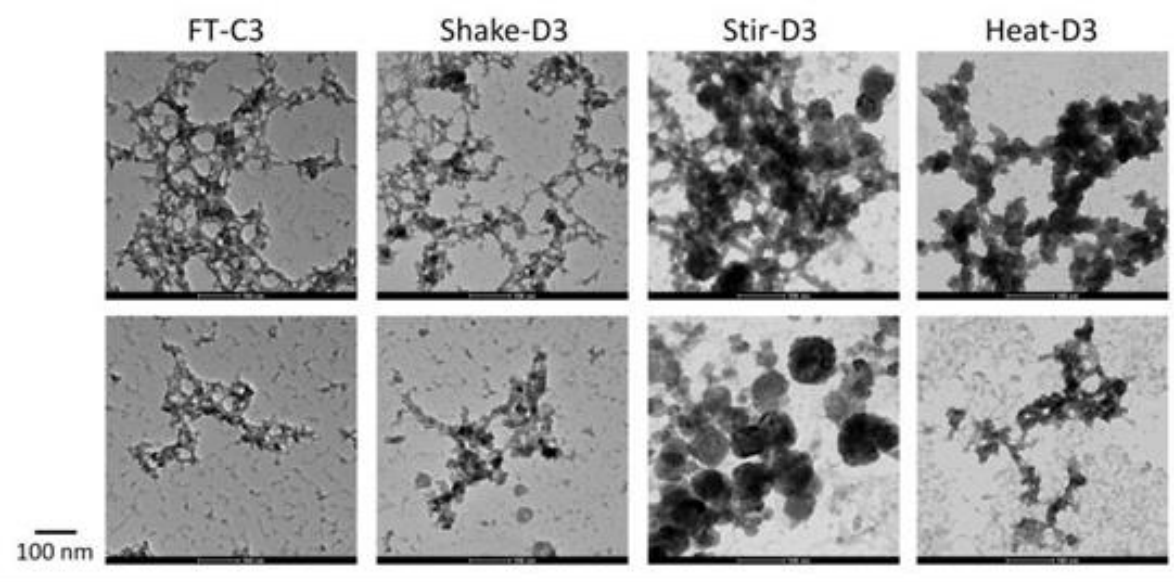
**During these studies, several therapeutically relevant model compounds are included, yet we are open to test how well our CDs fit your particular monoclonal antibody or other type of protein.**

# Cyclodextrin – protein interactions

## Outcomes of protein aggregation

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

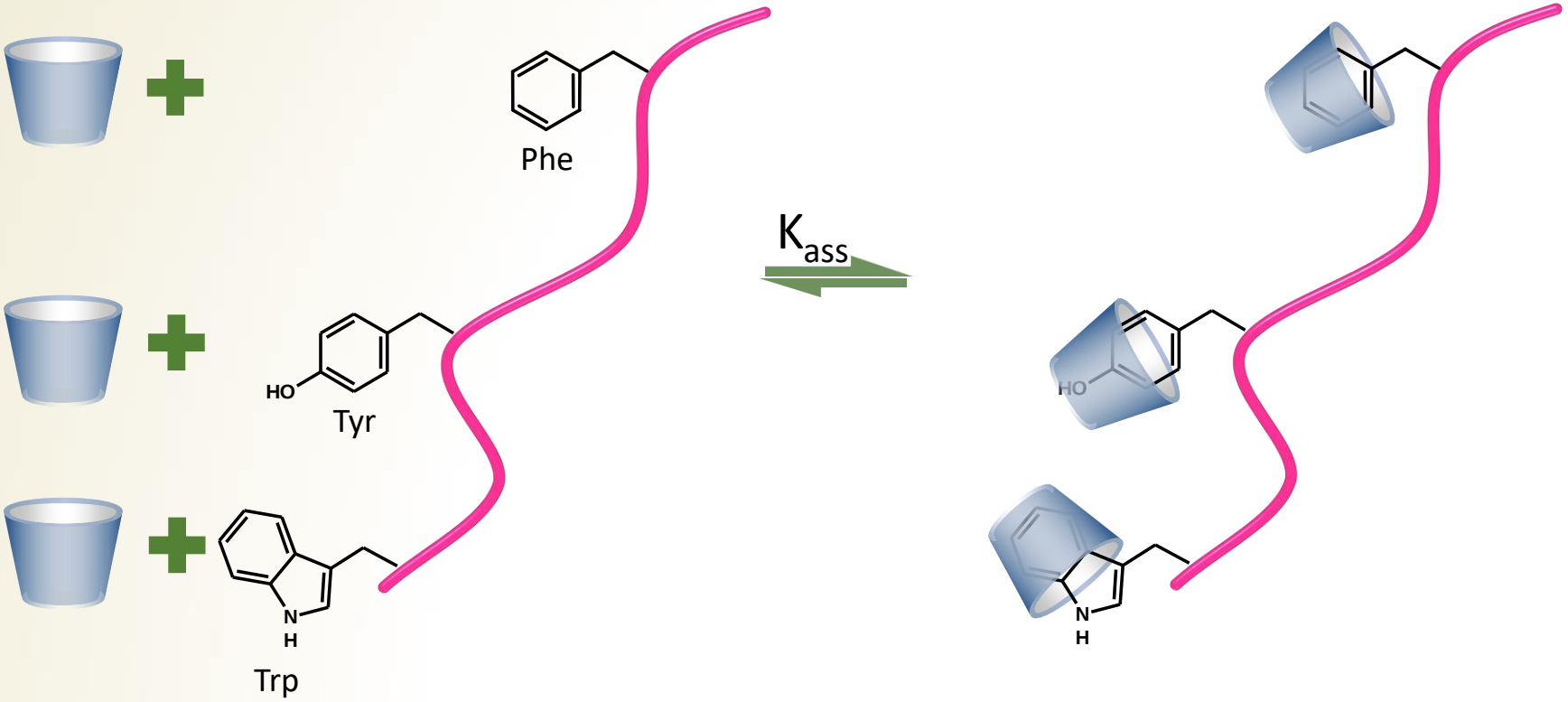
**mABs are particularly prone to aggregation**




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:

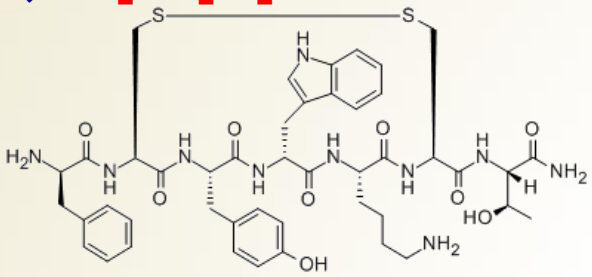
- CDs attract to hydrophobic regions of the proteins
- The interactions are host-guest and electrostatic type
- CDs act like artificial chaperons
- Certain CDs act like chaotropic agents and delay protein-protein interaction and thereby folding in solution

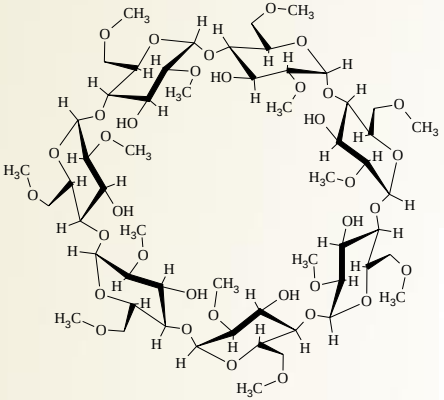
Han (2007), Serno (2010)

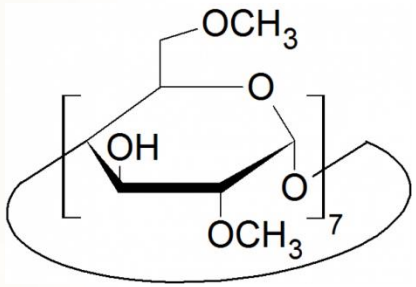
# Cyclodextrin – protein interactions

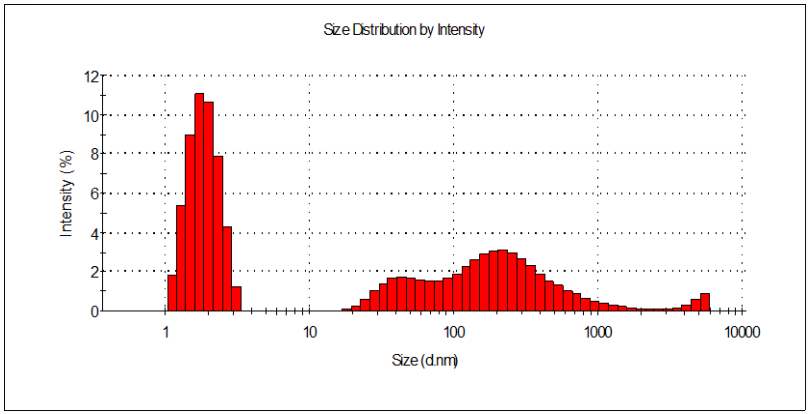
## Cyclodextrin's effect on peptide aggregation

**TT-232, heptapeptide**

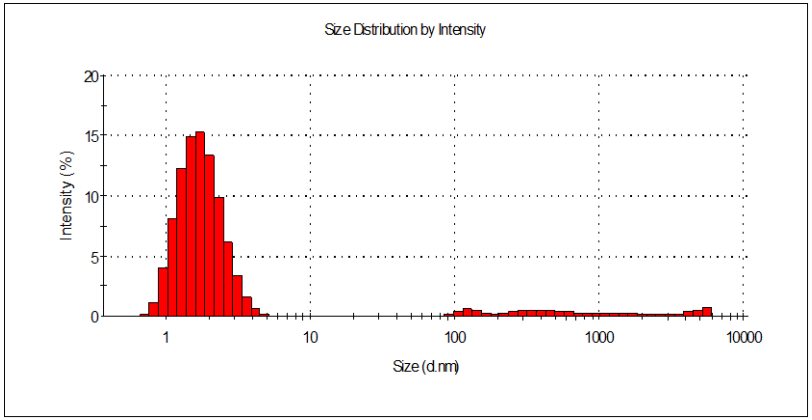







**Without additive**

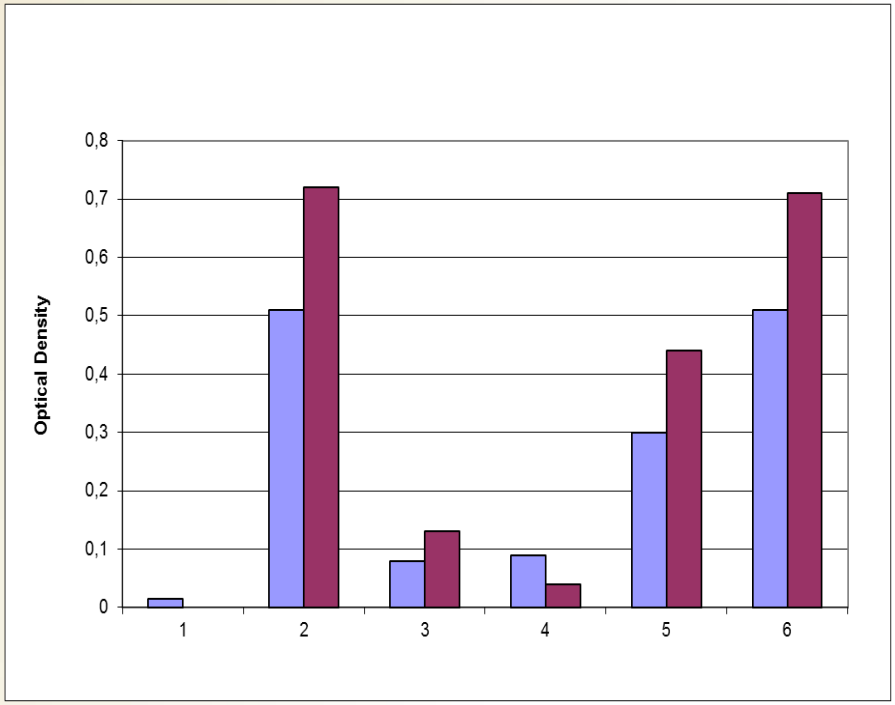


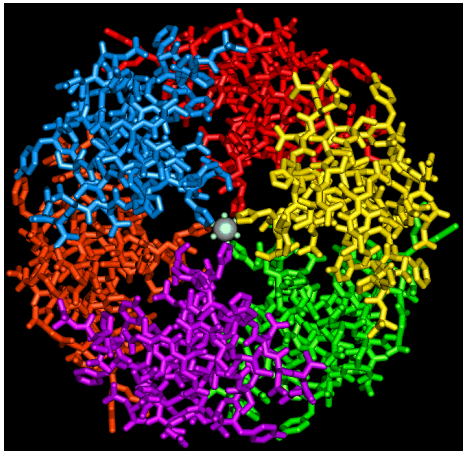

**With DIMEB-CD**

**Kéri (2007)**

# Cyclodextrin – protein interactions

## Cyclodextrin's effect on insulin aggregation



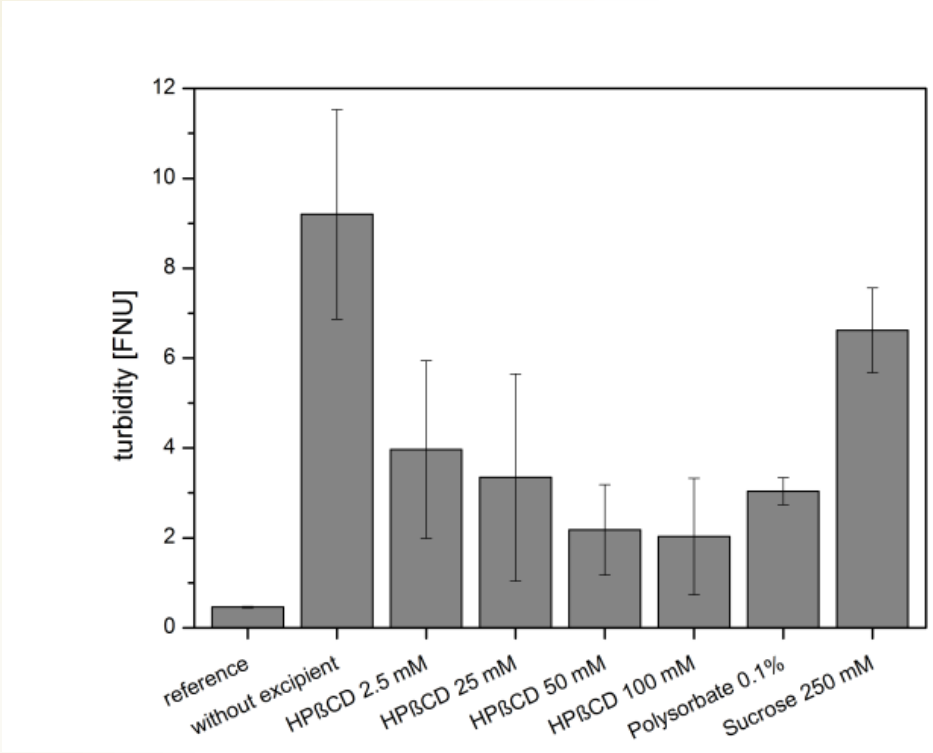
insulin hexamer

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

## Cyclolab offers:

- **Provide a cyclodextrin „starting kit” to evaluate the feasbility of CDs towards a particular problem**
- **Supply commercial quantities of any cyclodextrin during development and market phases**
- **Provide formulation, analytical or even custom synthesis services to establish a stable and efficient formulation with a target protein**