

# GETTING THE BEST OUT OF CYCLODEXTRINS

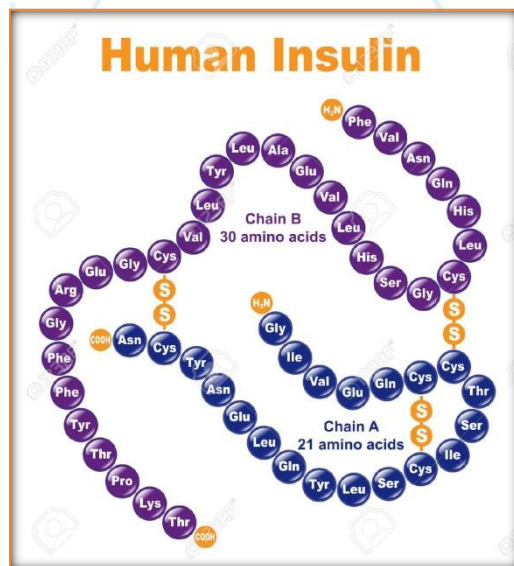
**Cyclodextrin enabled biologics**  
**A novel way of utilizing CDs**



**Cyclodextrins are molecular containers. As of 2020, 100+ products of **small molecules** are formulated with CDs.**

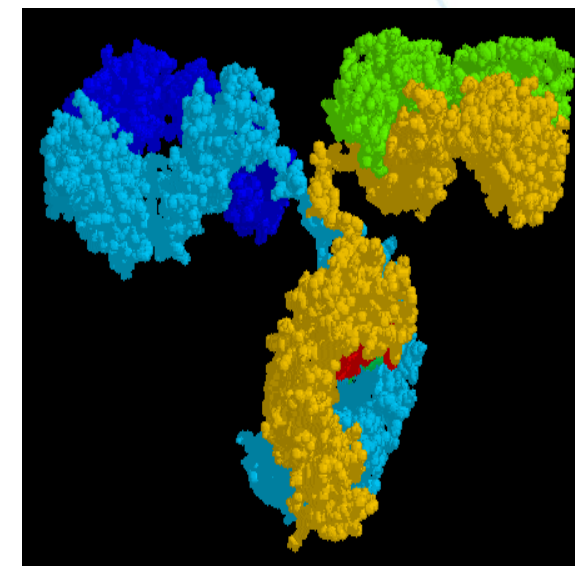


**Biological active substances (big molecules), proteins, peptides, monoclonal antibodies gained immense interest in product development recently**



Peptide hormone

5808 Da



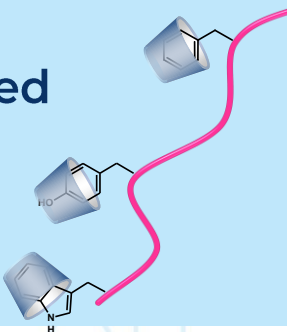
Monoclonal antibodies  
~1300 amino acids,

150 000 Da



### Why use CDs in protein and biological formulations?

- Safer than current excipients (e.g. Tween) – no peroxide formation, corresponding immunogenicity, degradation
- Prevention of aggregation, delayed folding
- Less protein adsorption onto container surface
- Reduced/maintained viscosity, improved injectability
- Life-cycle management



Protein without  
CD

Protein + CD1

Protein + CD2

Protein + CD3

Cyclodextrins' effect on  
stirring-induced aggregation  
of insulin

## Cyclodextrin – protein interactions

CycloLab has recently been putting huge 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.

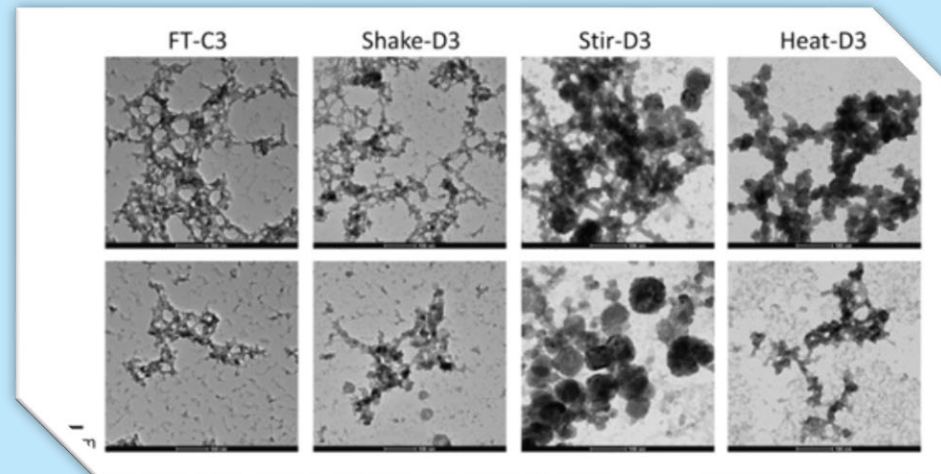


# Cyclodextrin – protein interactions

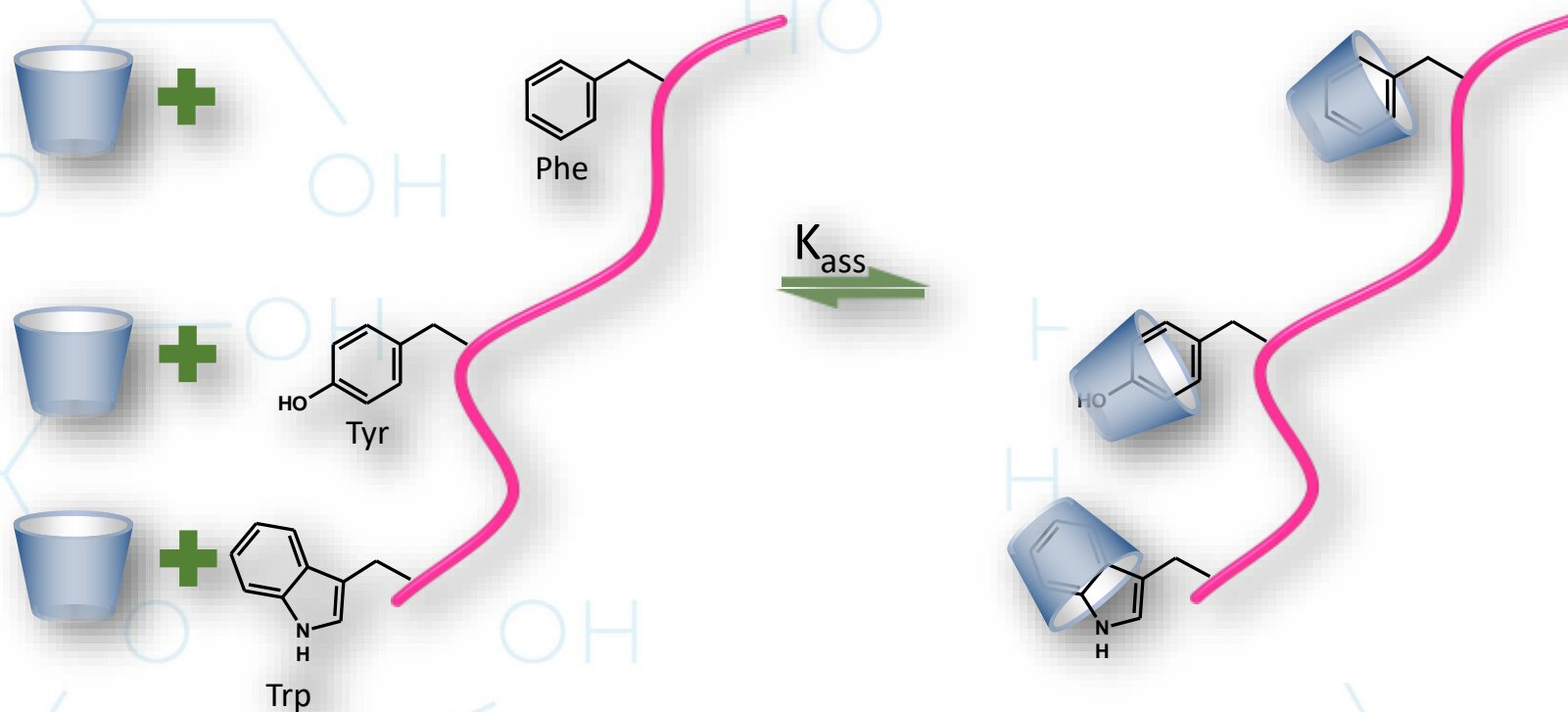
## Outcomes of protein aggregation

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

mABs are  
particularly  
prone to  
aggregation



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



## 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

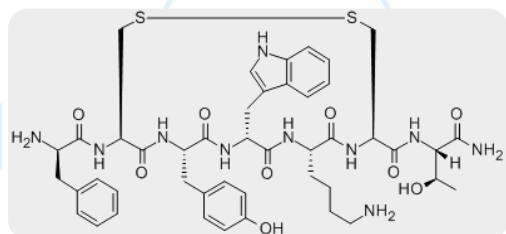
Certain CDs act like chaotropic agents and delay protein-protein interaction and thereby folding in solution



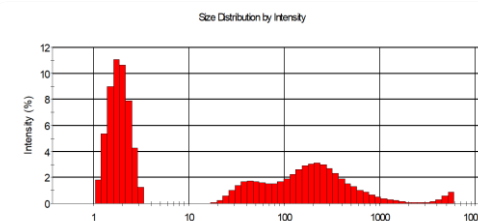
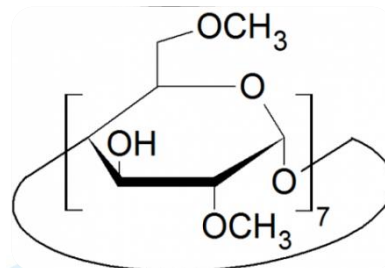
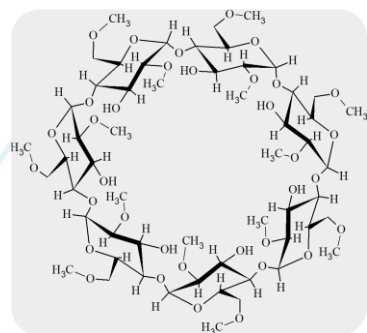


# Cyclodextrin – protein interactions

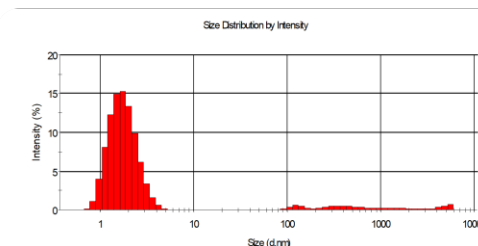
## Cyclodextrin's effect on peptide aggregation



TT-232, heptapeptide



Without additive

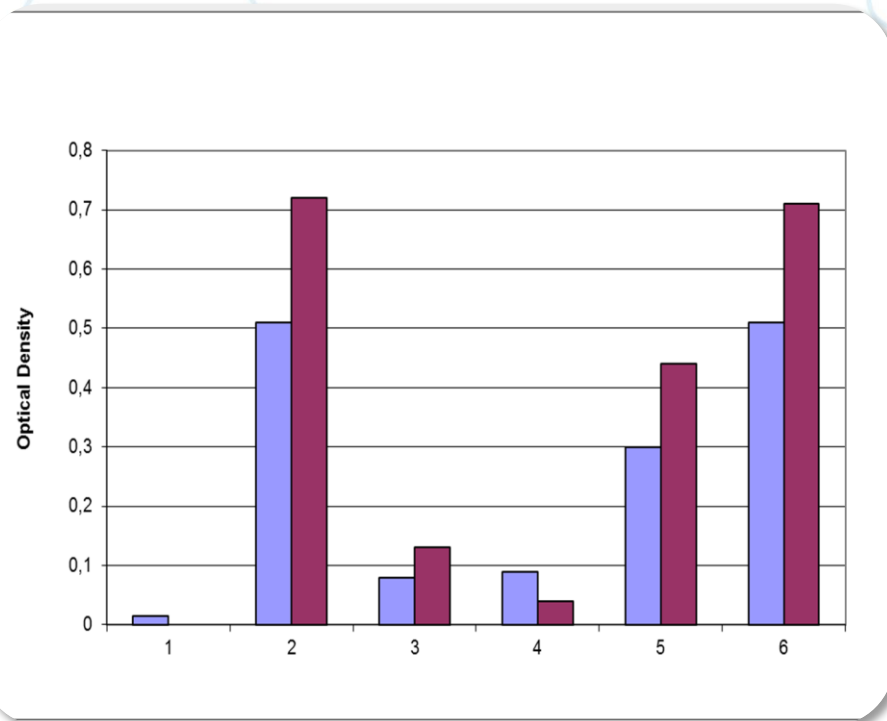


With DIMEB-CD

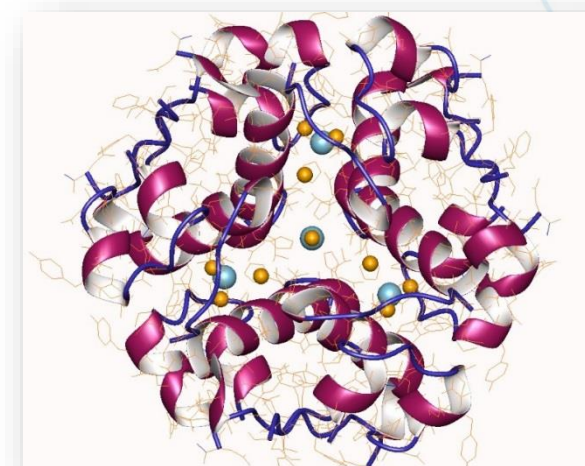
Kéri (2007)

# Cyclodextrin – protein interactions

## Cyclodextrin's effect on **insulin** aggregation



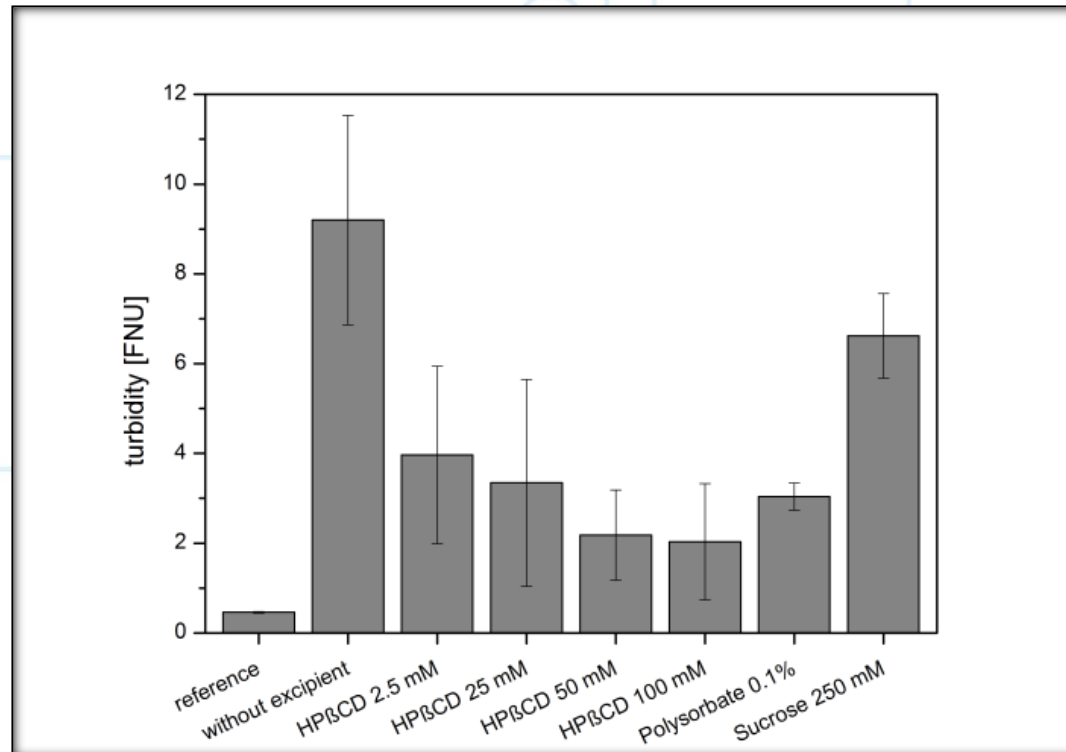
1. Control, 2. Stirred without CD,  
3. **HPBCD**, 4. **alpha-CD**, 5. **beta-CD**, 6. **gamma-CD**



insulin hexamer

## Cyclodextrin – protein interactions

### Cyclodextrin's effect on IgB aggregation



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

### Cyclodextrins or TWEEN?

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





# 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, <b>polysorbate 80</b> , 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, <b>polysorbate 80</b> and water for injections
Herceptin	trastuzumab	4 years at 2 °C – 8 °C.	histidine hydrochloride, histidine, trehalose dihydrate, <b>polysorbate 20</b>
Vetsulin	porcine insulin zinc suspension	<b>42 days</b>	zinc chloride, methylparaben, sodium chloride, sodium acetate, water
NovoLog	insulin aspart	<b>28 days</b>	glycerin, phenol, metacresol, zinc, disodium hydrogen phosphate dihydrate, sodium chloride and water for injection
ORTHOCLONE	muromonab-CD3	<b>9 months</b>	sodium phosphate, monobasic sodium phosphate, dibasic sodium chloride <b>polysorbate 80</b> water
Oncaspar	pegaspargase	<b>8 months (2-5°C)</b>	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

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

**Consequence:**

**mAb-mAb aggregation increases**

**Result: mAb-mAb**

**aggregation decreases**

## Vaccine adjuvants

### Hydroxypropyl betadex is a component in J&J COVID-19 vaccine

Janssen incorporated HPBCD into their monovalent Covid-19 vaccine composed of a recombinant, replication-incompetent adenovirus type 26 (Ad26) vector, constructed to encode the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) spike protein.

In the patent application of Janssen's HIV vaccine candidate (WO2017216288) HPBCD is applied in 4-6% (w/w) in the product.

Reason: HPBCD acts as a cryoprotectant, yet can also contribute to protein stabilization hindering aggregation and adsorption onto the container wall.

*Johnson & Johnson*



*Johnson & Johnson*



### CycloLab offers to

- Provide a cyclodextrin „starting kit” to evaluate the feasibility 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





# Cyclodextrin – protein interactions

## COMPANY CONTACTS

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