University of Pittsburgh

β-Cyclodextrin-threaded Biocleavable Polyrotaxanes Ameliorate Impaired Autophagic Flux in Niemann-Pick Type C Disease

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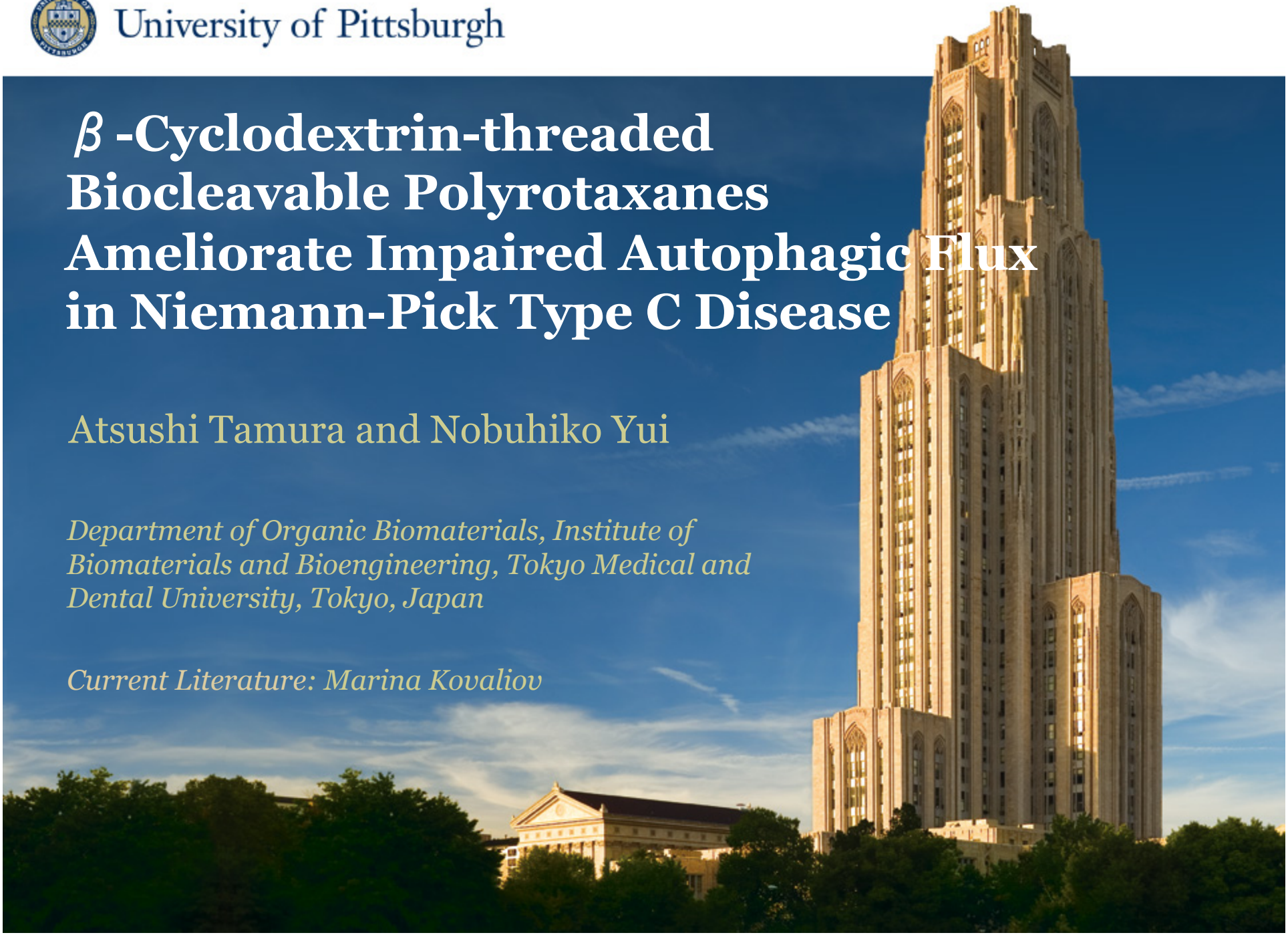
Current Literature: Marina Kovaliov



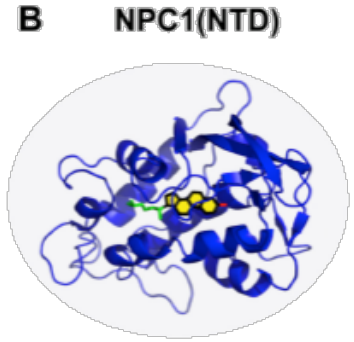
Niemann-Pick Type C Disease

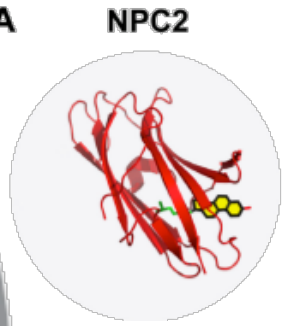
- **NPC is one of lysosomal storage disorders (LSDs)**
  - NPC is a genetic, pediatric, neurodegenerative disorder
  - Causing progressive deterioration of the nervous system, because of the cells inability to metabolize cholesterol
  - Affect children (4-7 years old) by accumulating with large amounts of cholesterol within the liver, spleen and **brain**

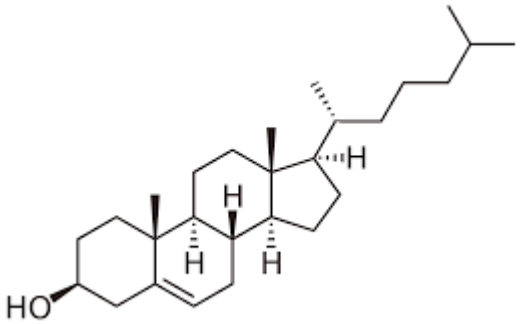
Vanier at al, (**2003**) Clin. Genet. 64, 269
Chang at al, (**2005**) J. Biol. Chem. 280, 20917

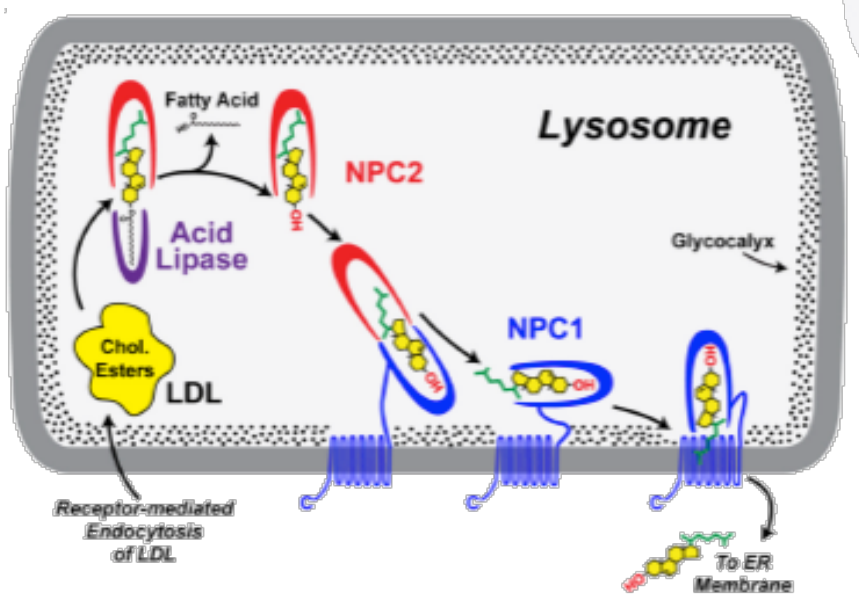
NPC1/NPC2 function

- **NPC disease caused by mutation of NPC1**
- NPC1 is intraellular cholesterol transporter
- NPC1 and NPC2 function as the cellular 'tag team duo' to catalyze the mobilization of cholesterol within the multivesicular environment of the endosomes/ lysosomes through the limiting bilayer to the ER and plasma membrane
- A dysfunction in NPC1 cause lysosomal accumulation of unesterified cholesterols

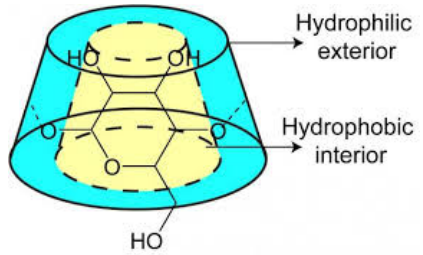








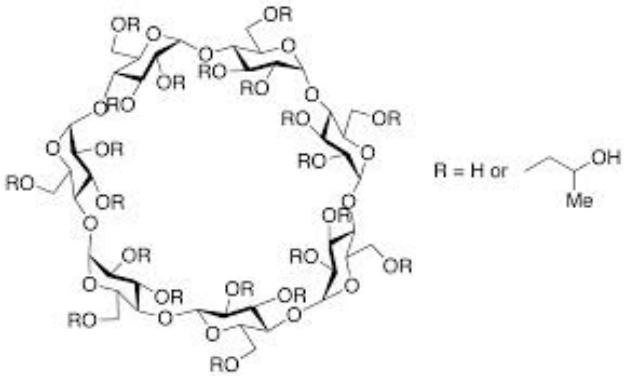
Experimental Use of 2-Hydroxypropyl-β-Cyclodextrin



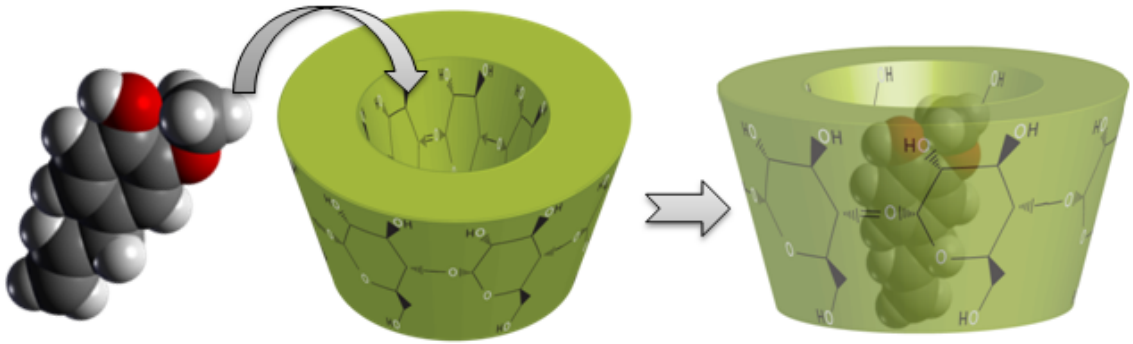
- Cyclodextrins (CD) have been shown to reduce symptoms and extend lifespan in animal models of the disease.
- Researchers at the Medical Center of University of Texas found, when NPC mice were injected with HP-β-CD, marked improvement in liver function tests, much less neurodegeneration, and, ultimately, significant prolongation of life occurred.
- In 2009, the FDA approved investigational new drug applications for Hempel twins, to receive intravenous infusions of HP-β-CD. The twins received HP-β-CD, 2500 mg/kg, administered as an eight-hour infusion, twice weekly.
- On September 20, 2011, the European Medicines Agency granted HP-β-CD orphan drug status and designated the compound as a potential treatment for NPC disease.

Liu at al, (**2009**) Proc. Natl. Acad. Sci. U.S.A. 106, 2377
Davidson at al, (**2009**) PLoS One 4, e6951
Porter at al. (**2010**). Sci. Transl. Med. 2, 56

HP-β-Cyclodextrin



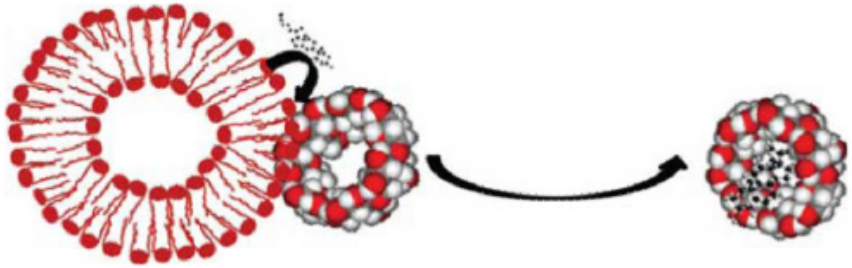
- Cyclodextrins (CD) are cyclic oligosaccharides shaped like hollow truncated cones.
- The exterior of the cone is hydrophilic and the interior hydrophobic, imparting the ability to bind small hydrophobic molecules such as cholesterol in the interior, thereby solubilizing them in aqueous media.
- Cyclodextrins can have over 15 glucopyranose units per ring; derivatives of β-cyclodextrin, containing seven units, are most widely used in pharmaceuticals because of their high affinity for hydrophobic compounds, low toxicity, and price.



McCauliff et al, (**2011**) Biochemistry. 50(34): 7341

Mechanism of HP-β-CD

- The mechanisms of action of HP-β-CD remain unclear. Although HP-β-CD cannot cross membranes, it can be delivered via pinocytosis to late endosomes/lysosomes, where it can replace the function of NPC1 and NPC2 proteins and promote cholesterol esterification by cholesterol acyl transferase (Co-A).
- Another hypothesis proposes that HP-β-CD increases the chemical activity of plasma membrane cholesterol, facilitating its movement from endosomes and lysosomes.



McCauliff et al, (**2011**) Biochemistry. 50 (34) 7341

β -CD-Threaded Biocleavable Polyrotaxanes

- Polymeric supermolecules composed of cyclic compounds threaded onto a linear polymer chain, for the lysosome-specific delivery of β -CD

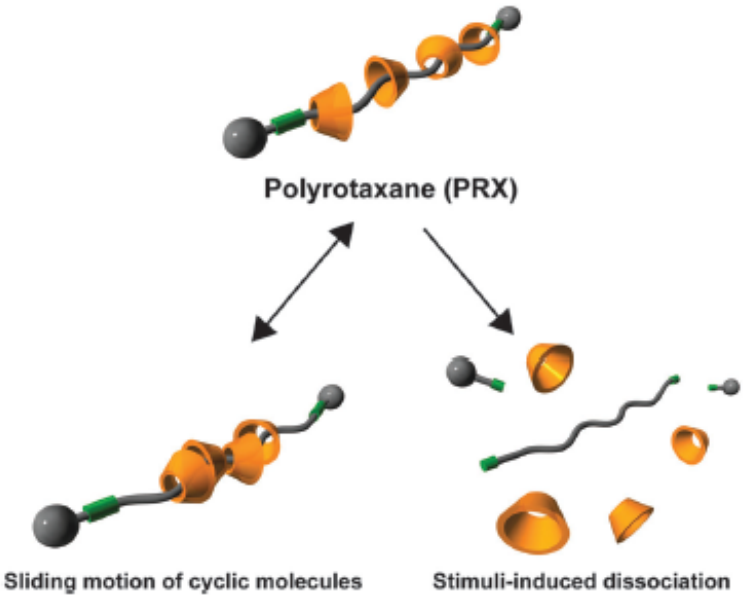
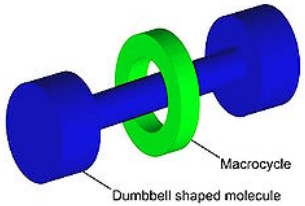


Fig. 1 Schematic illustration of polyrotaxanes (PRX) showing the sliding motion of cyclic molecules along a polymer axle and stimuli-induced supramolecular dissociation *via* the cleavage of bulky stopper molecules.

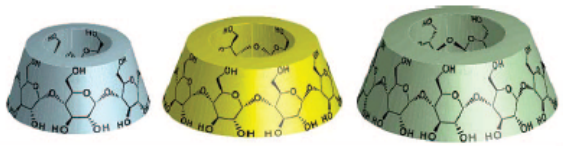
Polyrotaxane



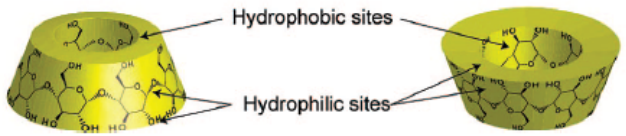
Harada at al, *Chem. Rev.* ***2009, 109, 5974–6023***

- A **rotaxane** is a mechanically-interlocked molecular structure consisting of a "**dumbbell shaped molecule**" which is threaded through a "**macrocycle**".
- The two components of a rotaxane are kinetically trapped since the ends of the dumbbell (stoppers) are larger than the internal diameter of the ring and prevent dissociation of the components since this would require significant distortion of the covalent bonds.
- **Polyrotaxanes** are molecular assemblies, in which many macrocycles are mechanically interlocked on a dumbbell-shaped molecule by bulky stoppers on its ends.

Table 1. Chemical Structure and Properties of Cyclodextrins (CDs)



	α-CD	β-CD	γ-CD
Molecular Weight	972	1135	1297
No. of Glucose Units	6	7	8
Cavity Diameter (nm)	0.47	0.60	0.75
Height of Torus (nm)	0.79	0.79	0.79



incorporating PEG and α-CD.

Polyrotaxane

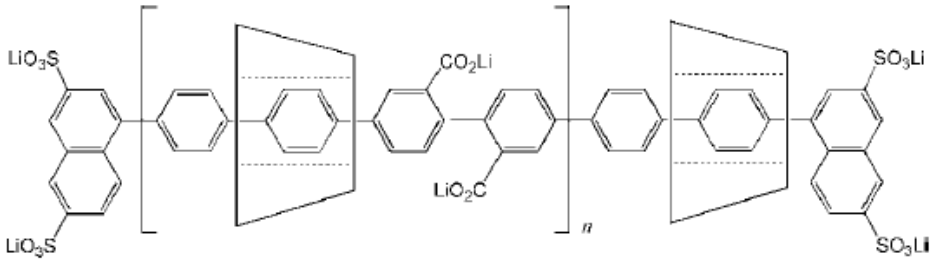
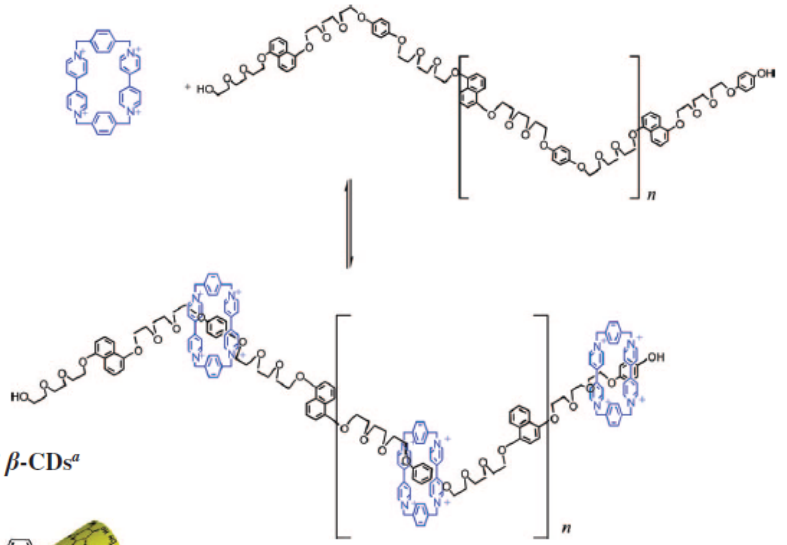
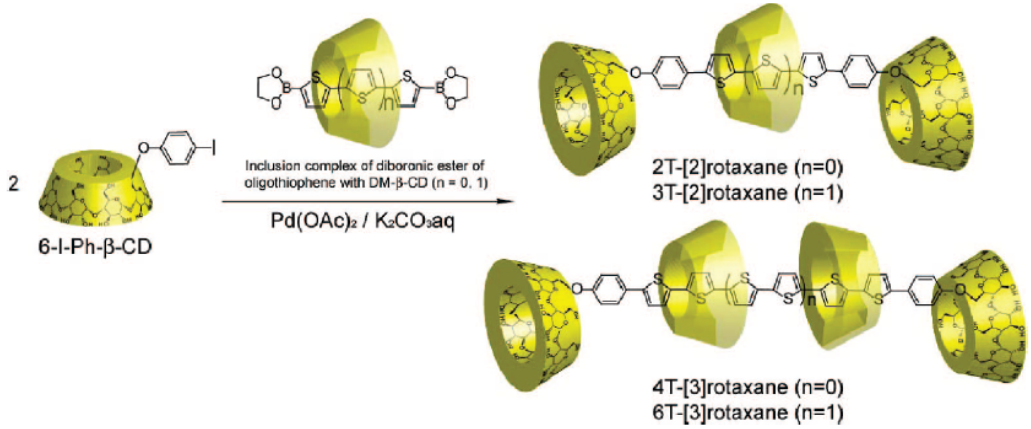


Figure 24. Polyrotaxane prepared from β-CD and poly(*p*-phenylene).

Scheme 12. Formation of the Poly*pseudo*rotaxane from Cyclobis(paraquat-phenylene) and a Polyether Derivative



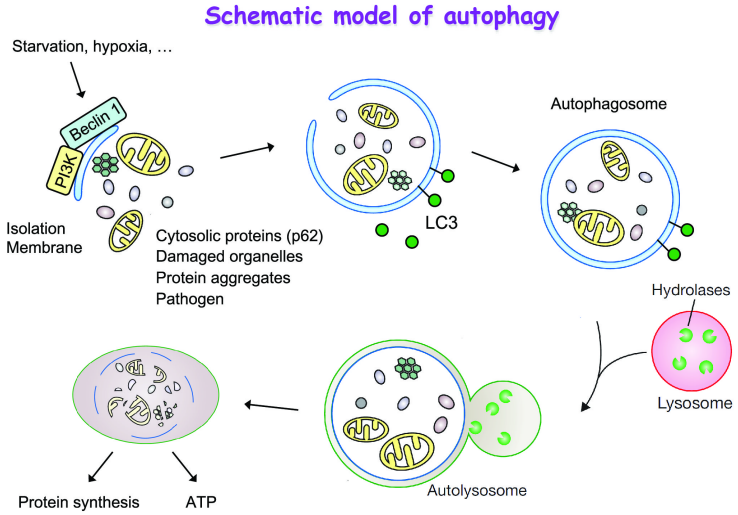
Scheme 1. Preparation of DM-β-CD-oligothiophene Based Rotaxanes with the End Stopper of β-CDs[a]



Harada at al, (**2009**) Chem. Rev., 109, 5974–6023

Impaired Autophagy in NPC Disease

- In NPC disease, various researchers have reported autophagosome accumulation even in the basal condition .
- Autophagy is a bulk degradation system of cytoplasmic protein aggregates and subcellular organelles, is of increasing importance with respect to its relation to various diseases.
- An improvement in impaired autophagy is required for the treatment of NPC disease in addition to a chronic cholesterol accumulation in lysosomes.
- It has been reported that HP-β-CD induces the decline of autophagic flux in normal and NPC1 model cells due to lysosomal cholesterol accumulation inhibits the fusion of lysosomes and autophagosomes.



Previous Studies

Yui at al, (**2014**) Chem. Commun., 50, 13433

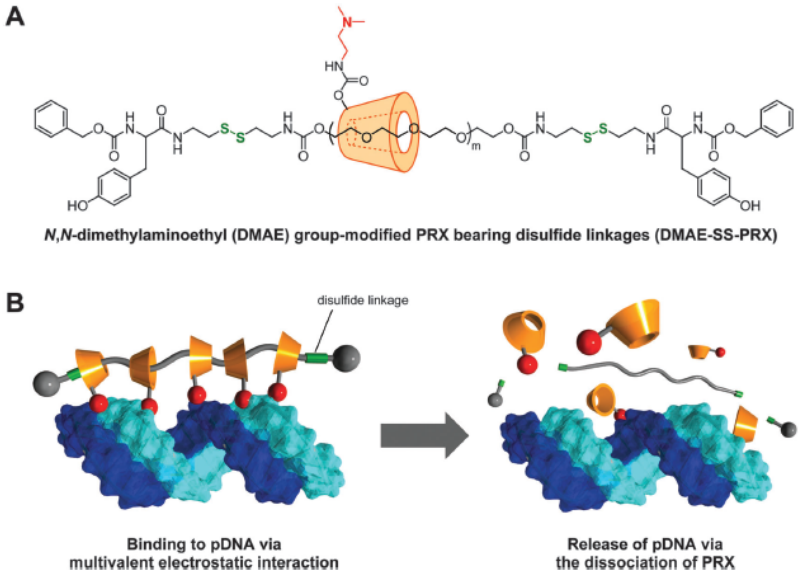
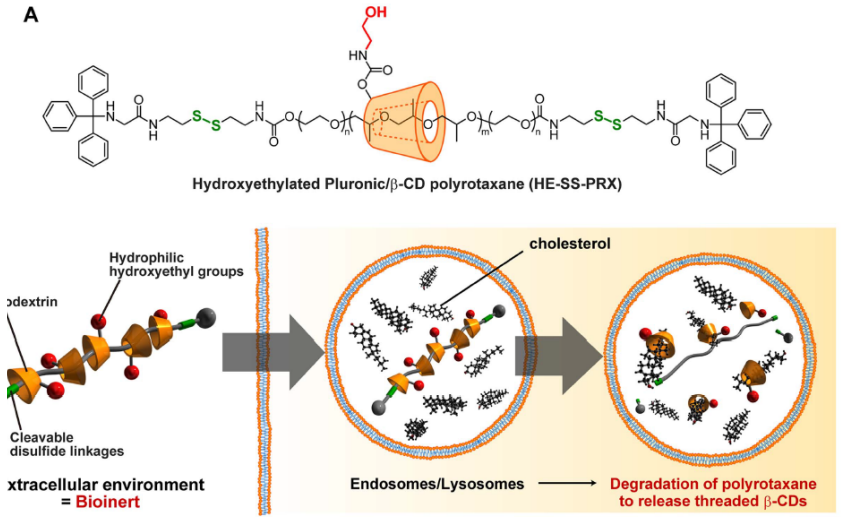


Fig. 4 (A) Chemical structure of *N,N*-dimethylaminoethyl (DMAE) group-modified PRX bearing terminal disulfide linkages (DMAE-SS-PRX). (B) Schematic illustration of an electrostatic interaction between DMAE-SS-PRX and pDNA, and the supramolecular dissociation *via* the cleavage of the terminal disulfide linkages in DMAE-SS-PRXs in an intracellular environment.

Yui, N at al, *(***2014**) *Scientific Reports*, 4, 4356



In This Study

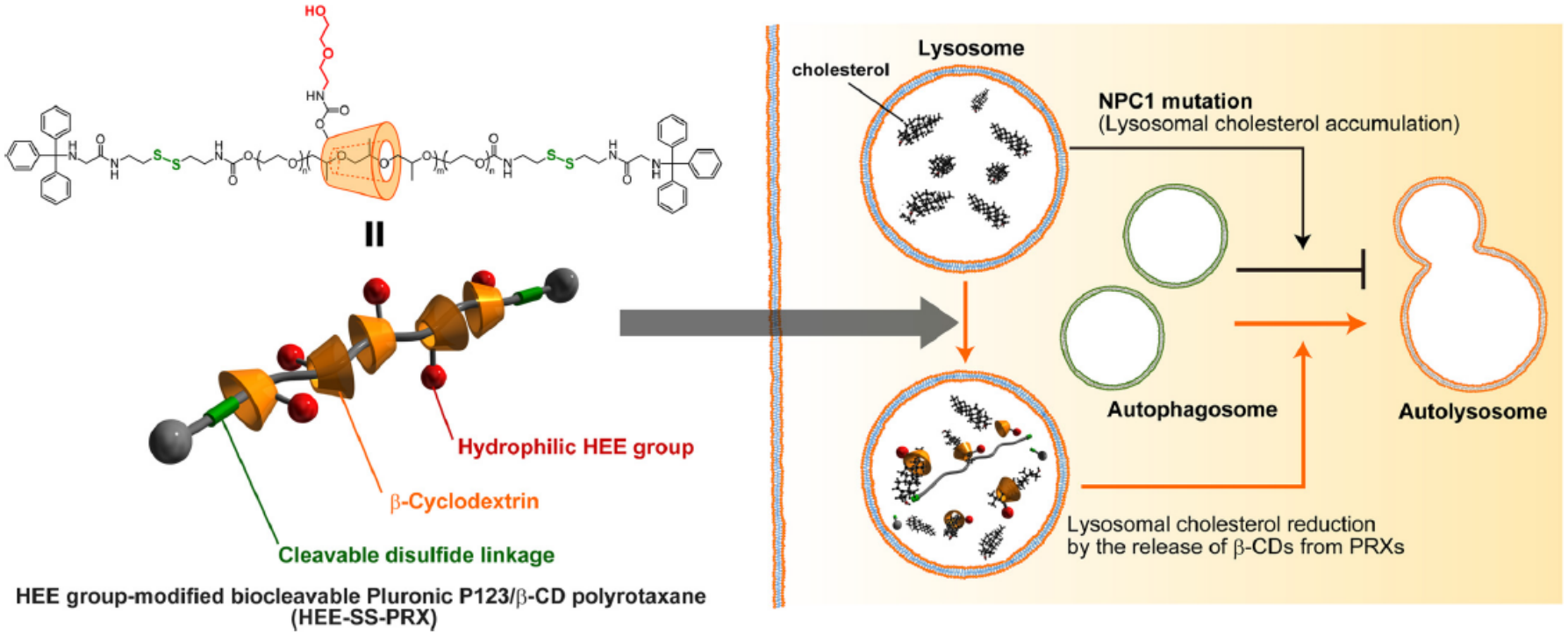
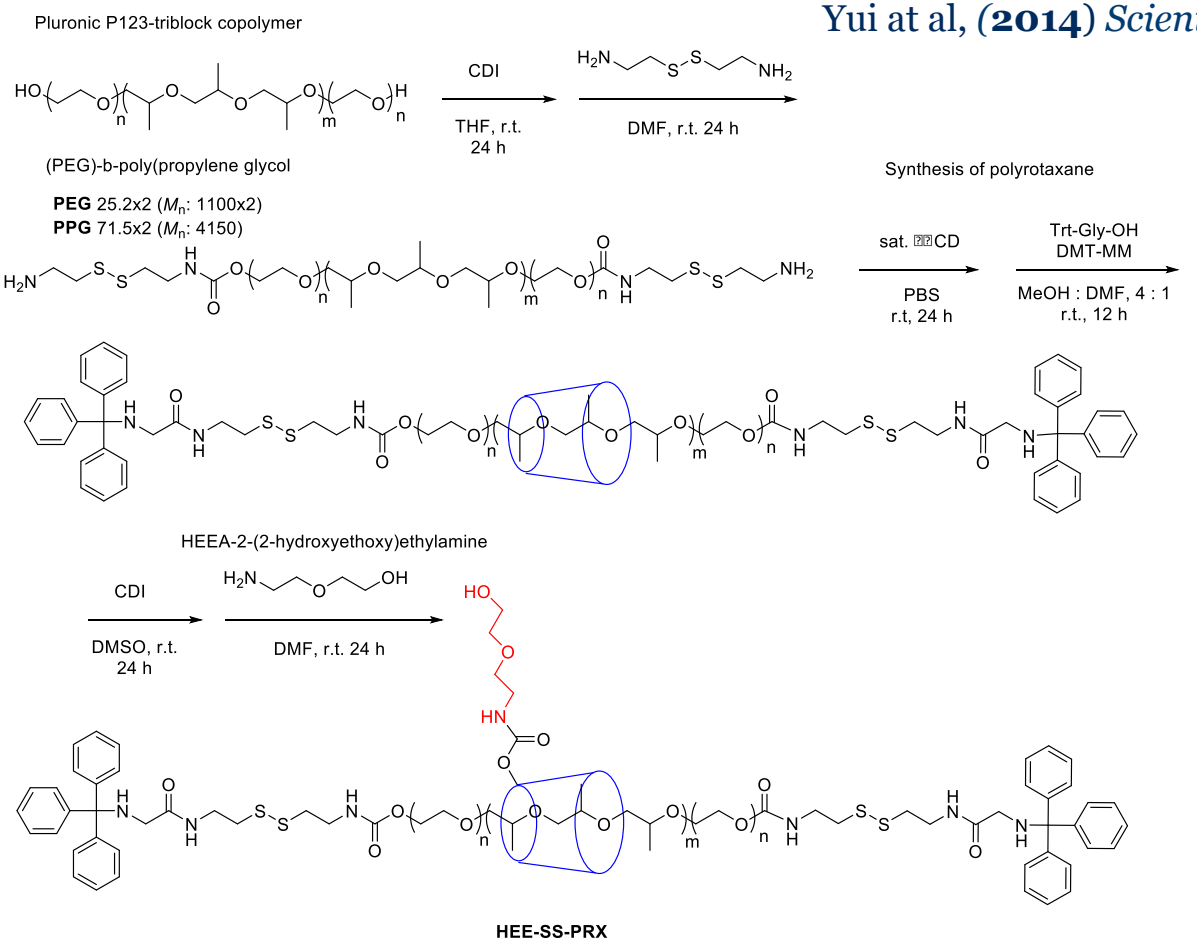


FIGURE 1. **Schematic illustration of β-CD-threaded biocleavable PRXs and the impaired autophagic flux in NPC disease.** The NPC1 mutation hinders the fusion of autophagosomes to lysosomes, whereas treatment with the biocleavable PRXs facilitates autolysosome formation.

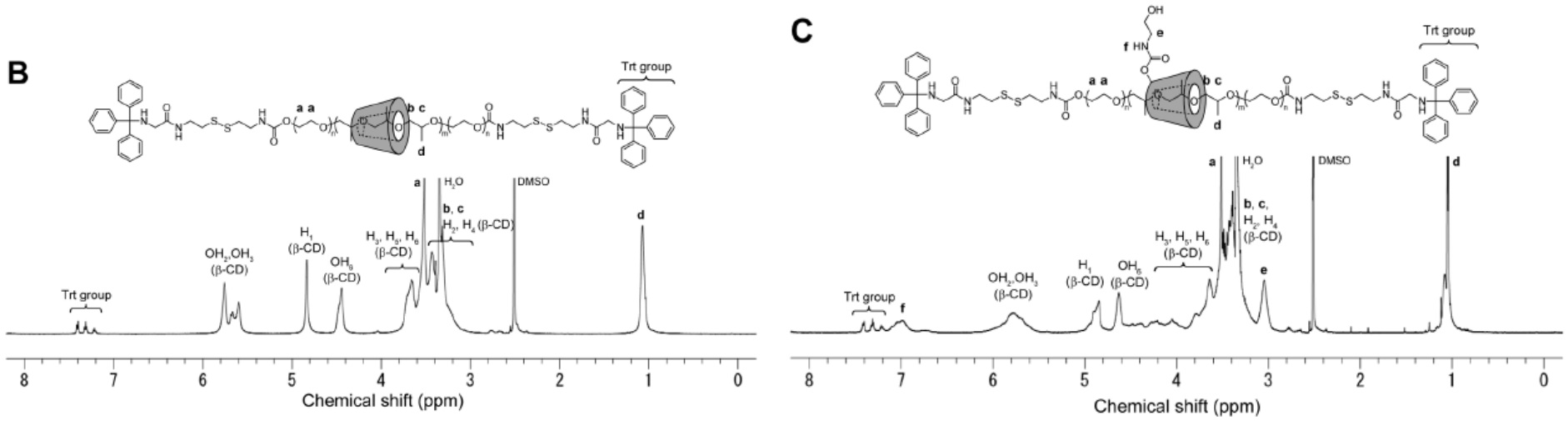
Synthesis of HEE-SS-PRX

Yui at al, (**2014**) *Scientific Reports*, 4, 4356



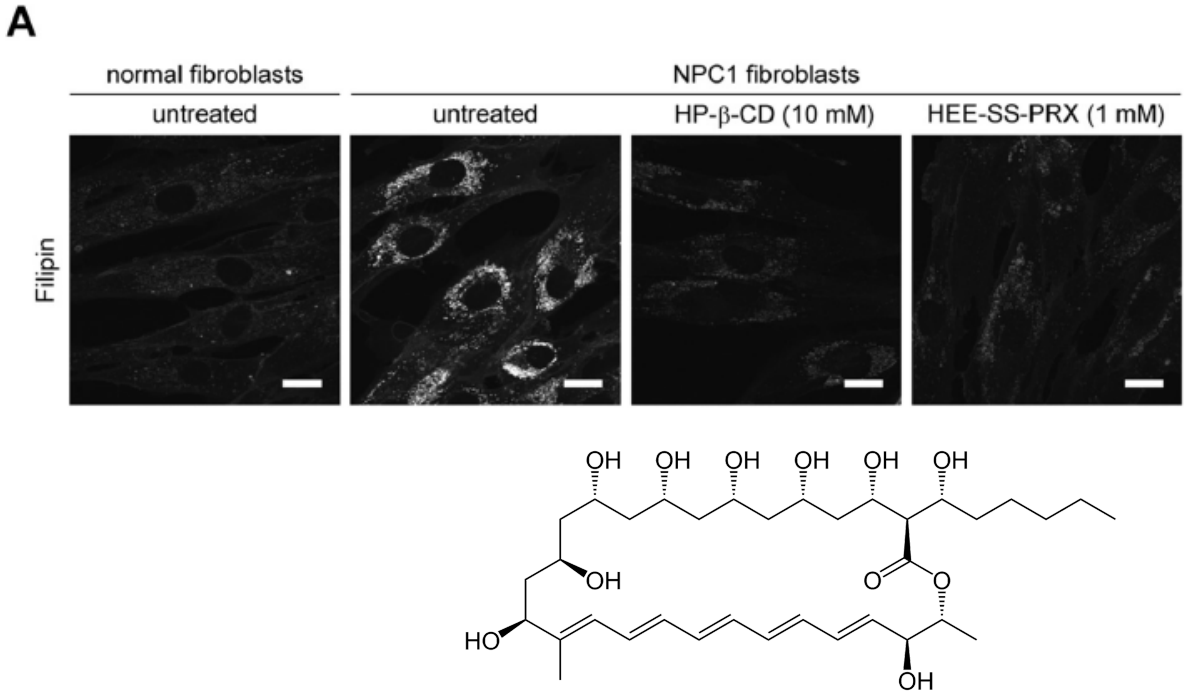
NMR Characterization

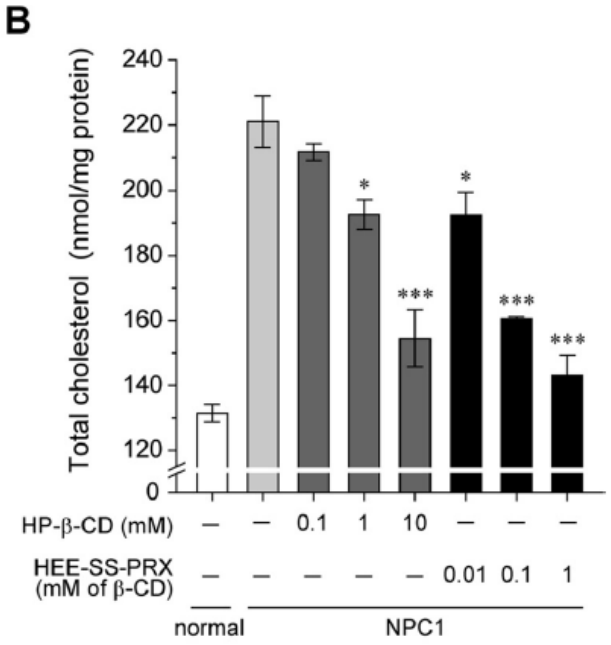
- The number of β-CDs threaded onto the P123 was determined from the 1H NMR peak area between 4.84 ppm (H1 proton of β-CD) and 1.06 ppm (-CH3 of the P123).



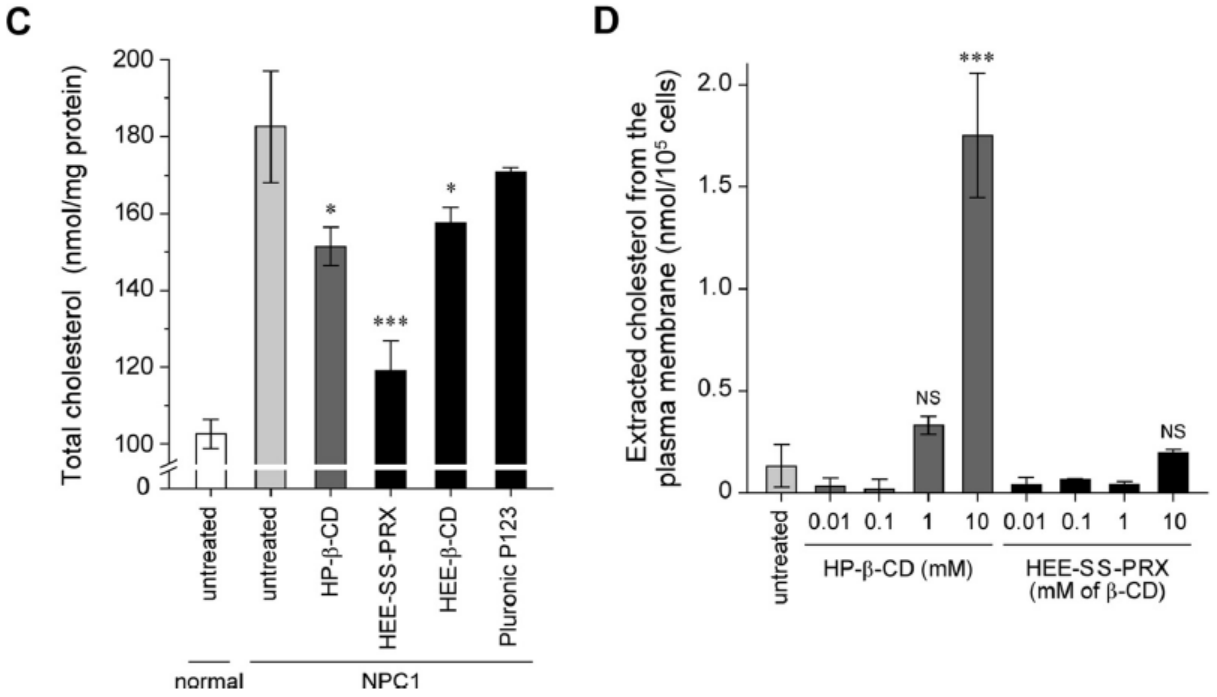
Cholesterol Reduction

- To confirm cholesterol reduction ability of biocleavable polyrotaxanes in NPC disease patient-derived fibroblasts, filipin staining for cholesterol and quantification of cholesterol were performed





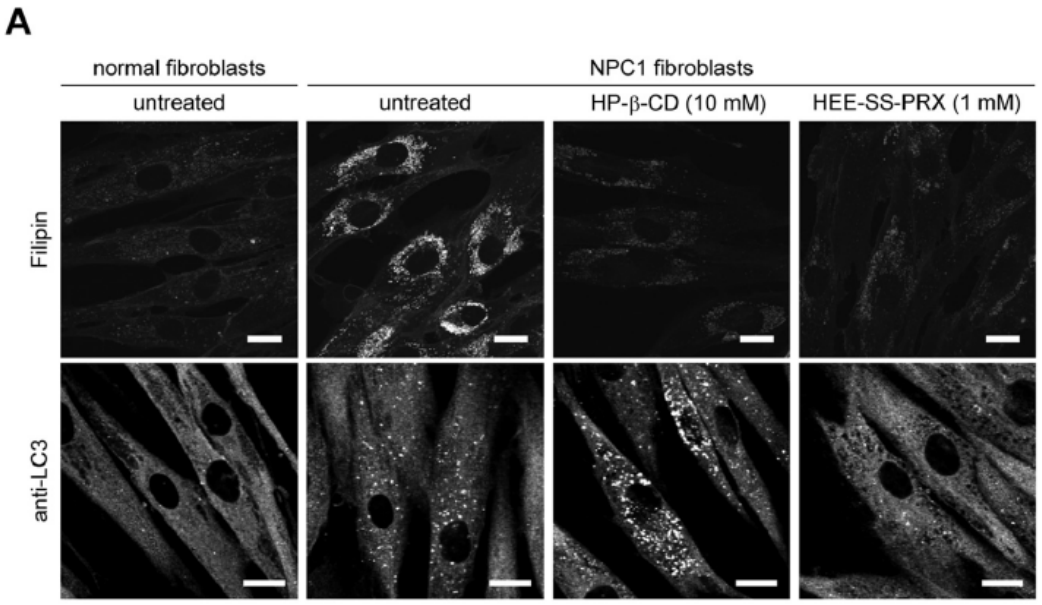
Cholesterol Reduction

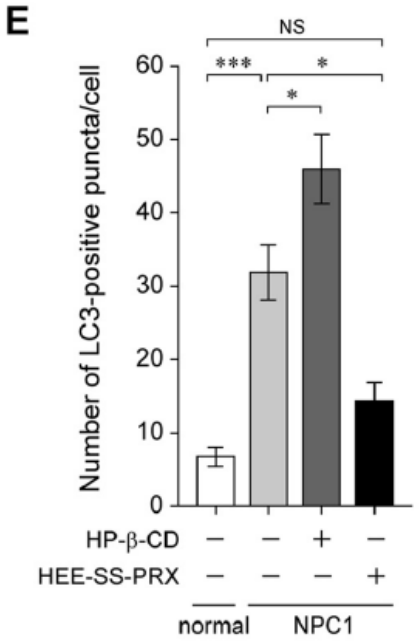


- HEE-SS-PRX reduced lysosomal cholesterol to a much lower concentration than HP- β -CD without cholesterol extraction from the plasma membrane

Autophagosomes Reduction

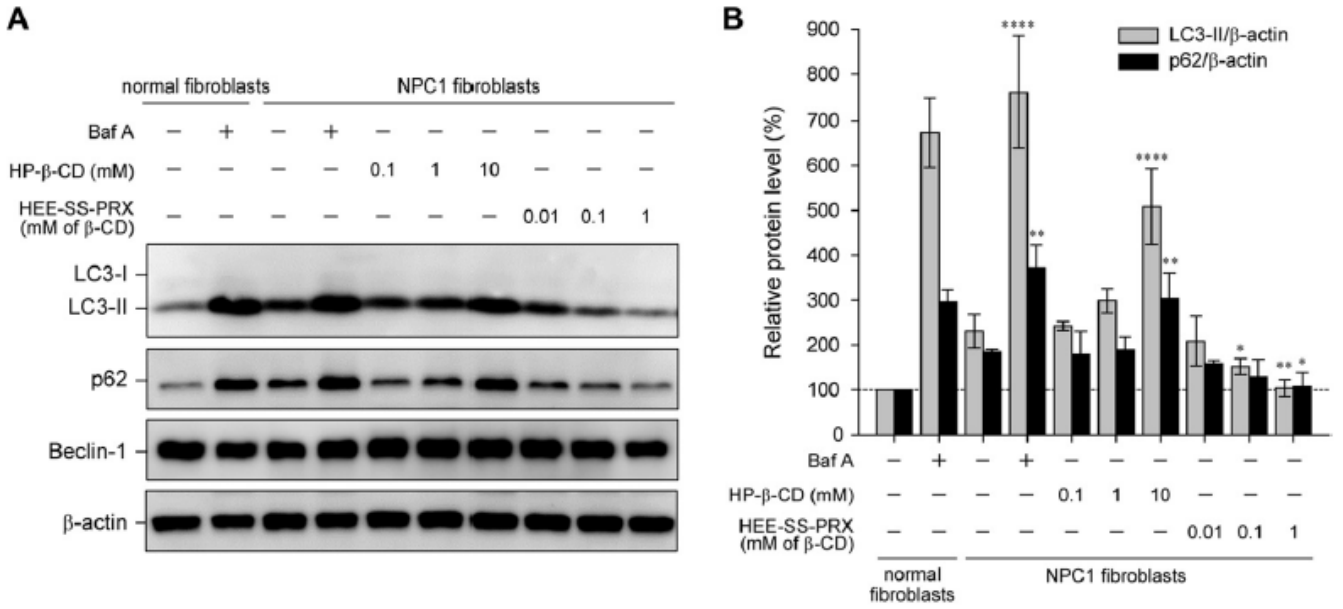
- The accumulation of autophgosomes in NPC1 fiproblasts was evaluated in immunostaning of microtubule-associated protein 1 light chain 3 (LC3) which is a soluble cytosolic protein (LC3-I form), whereas C-terminal modification with phosphatidylethanolamine (LC3-II form) leads to localization at the autophagosomal membrane.





Improvement of Autophagosomes and p62 Accumulation

- Further confirmation of these results was obtained by immunoblotting for LC3-II and p62/SQSTM1 (p62), a selective substrate for autophagic protein degradation
- Baf A, a specific inhibitor of the vacuolar-type H^+-ATPase, resulted in increased levels of both LC3-II and p62 in the normal and NPC1 fibroblasts due to the decrease in lysosomal enzymatic activity by the neutralization of the lysosomal pH



Conclusions

- HP- β -CD treatment induces the cytoplasmic accumulation of autophagosomes and perturbs the protein degradation, in contrast to β -CD-threaded PRXs (HEE-SS-PRX) that improved the impaired autophagic flux in NPC fibroblasts.
- HEE-SS-PRX had no effect on the lysosomal enzymatic activity it is reasonable to consider that the reduction of autophagosome accumulation could be due to the promotion of and the fusion of autophagosomes and lysosomes.
- HEE-SS-PRX can simultaneously improve lysosomal cholesterol accumulation and autophagy impairment in NPC disease.
- Therefore, β -CD-threaded biocleavable PRXs have great potential for the treatment of NPC disease.