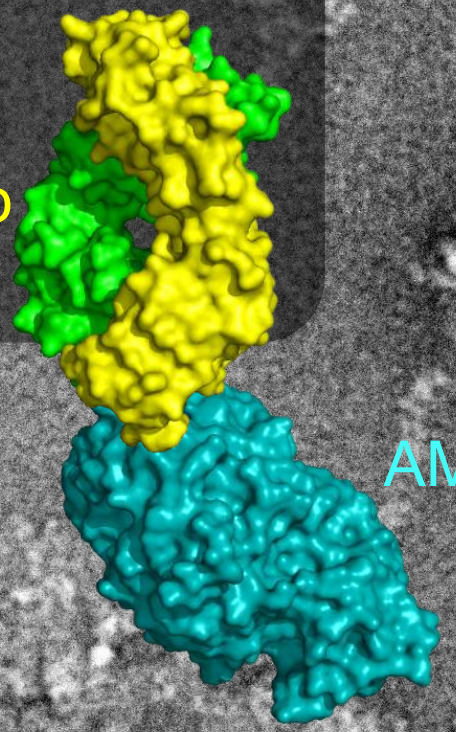
Characterization and degradation of Lafora Bodies for the treatment of Lafora disease

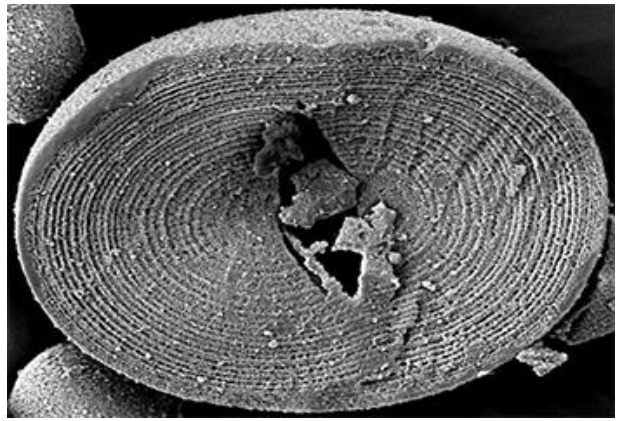
Katy Brewer & Tracy McKnight
Gentry lab & Valerion Therapeutics

valerion

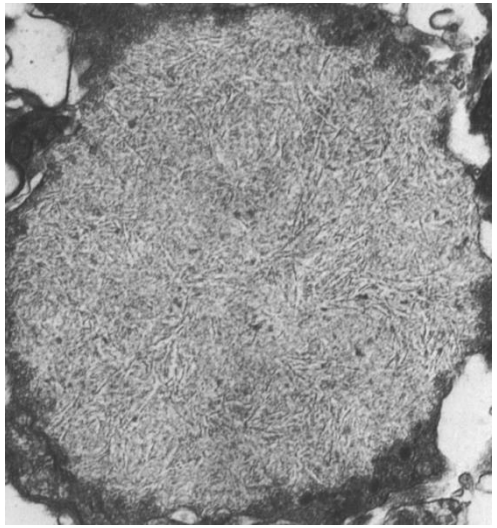


Corn starch and LBs are degraded by α-amylase

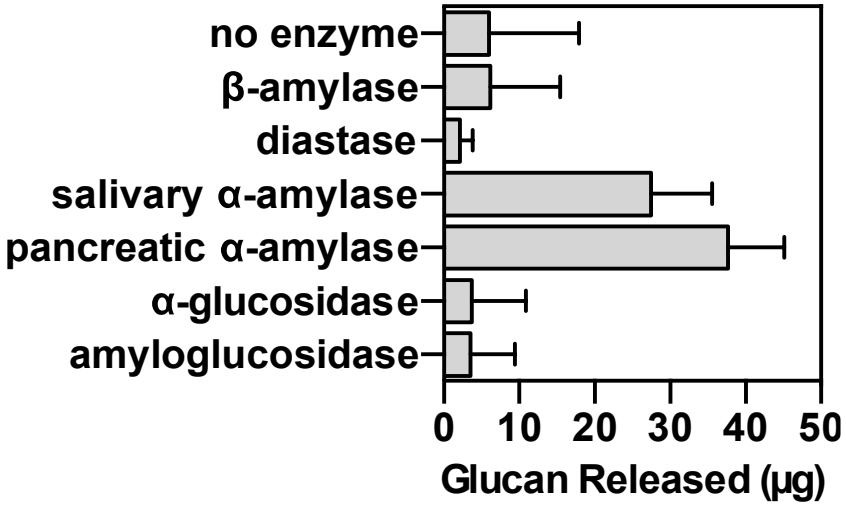
Starch (5-100 µm)

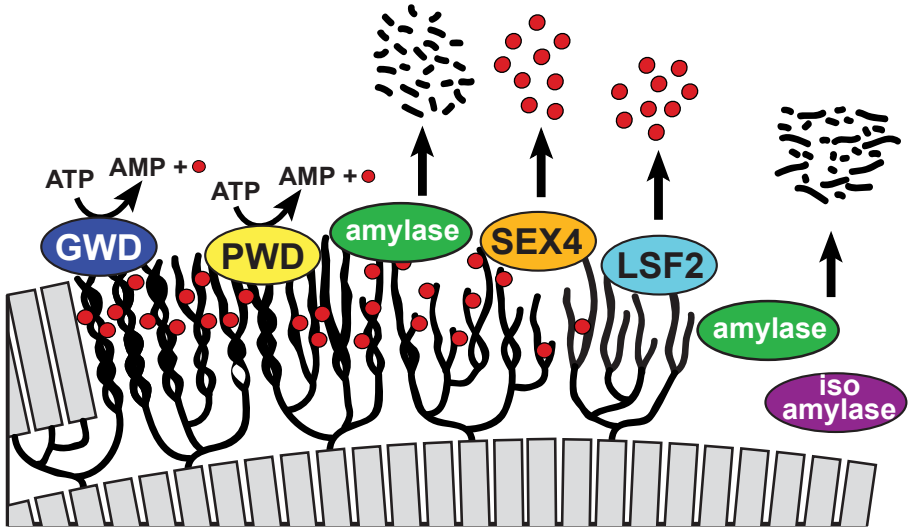


Lafora Body (2-40 µm)

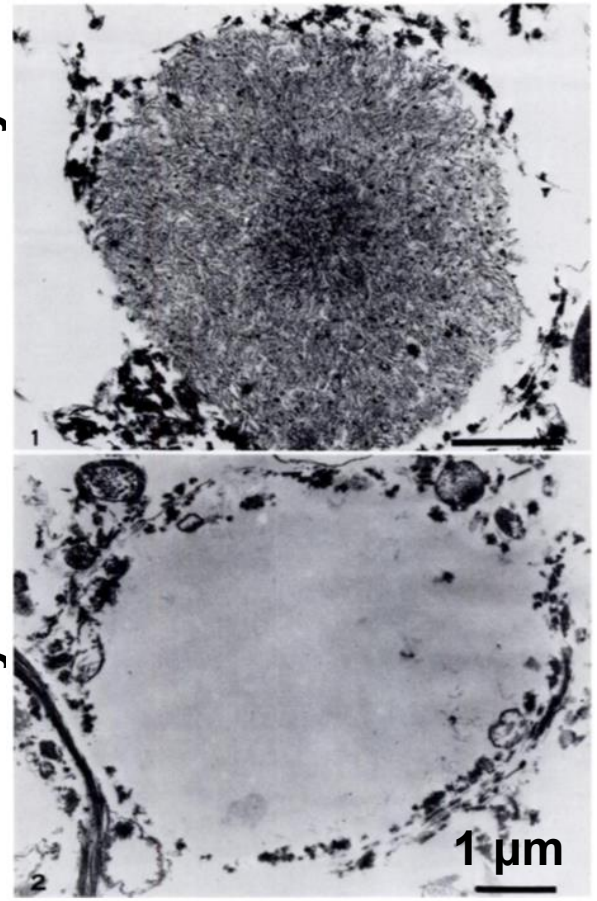


Berard-Badier et al., 1979.
Albrecht Von Graefes Arch Klin Expo Ophthalmol.



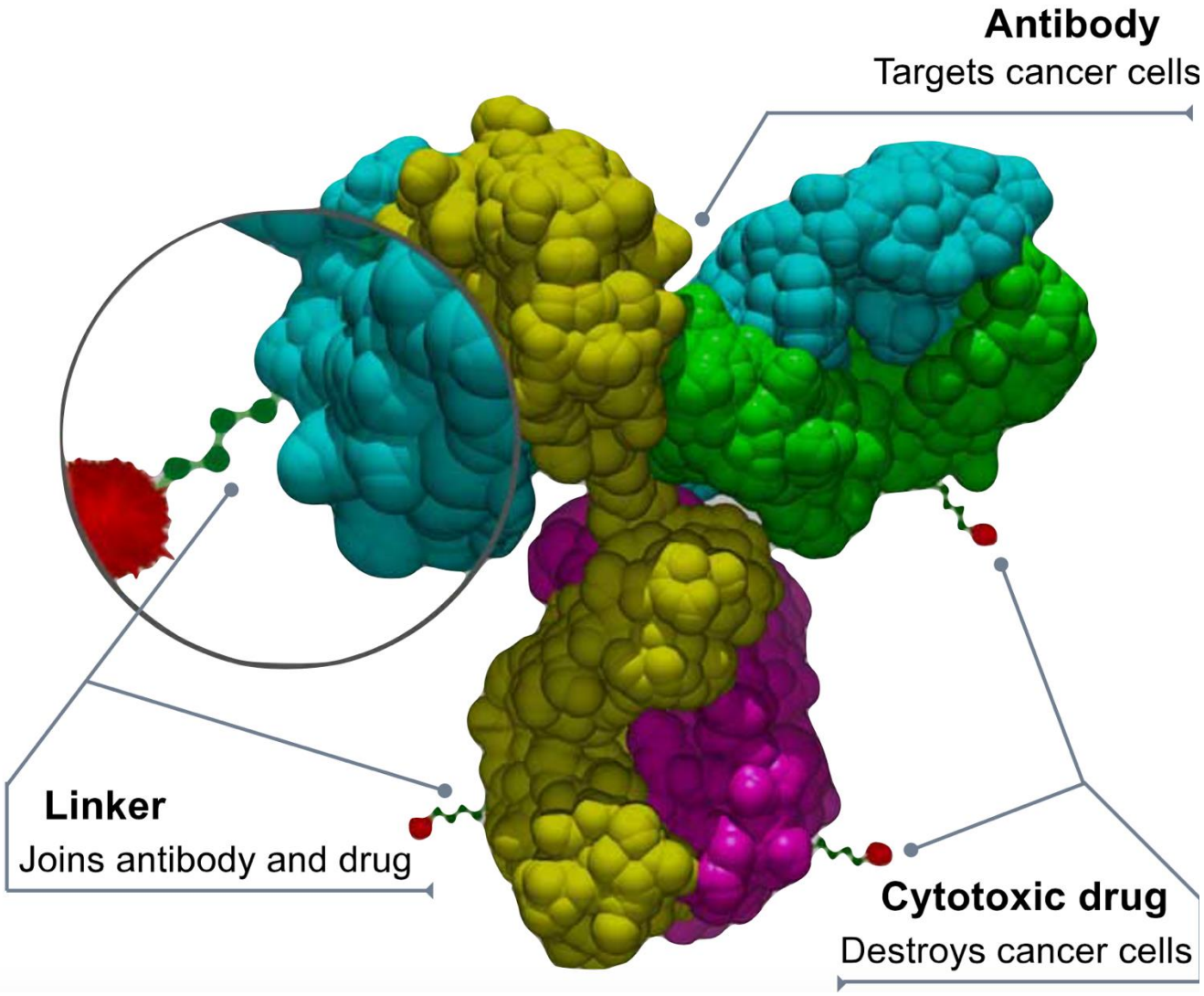


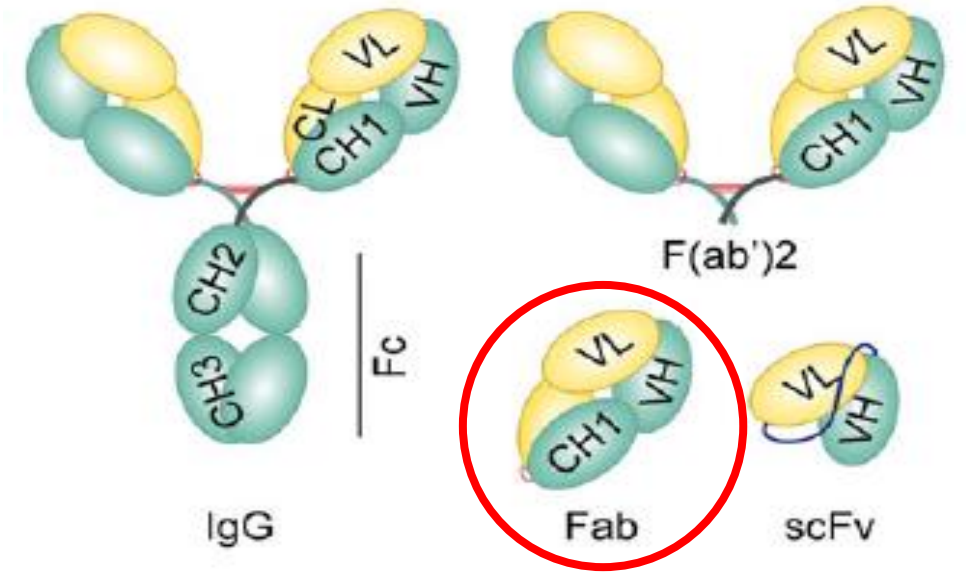
Lafora body (fixed human tissue)



Nikaido et al. *J Histochem Cytochem* (1971).

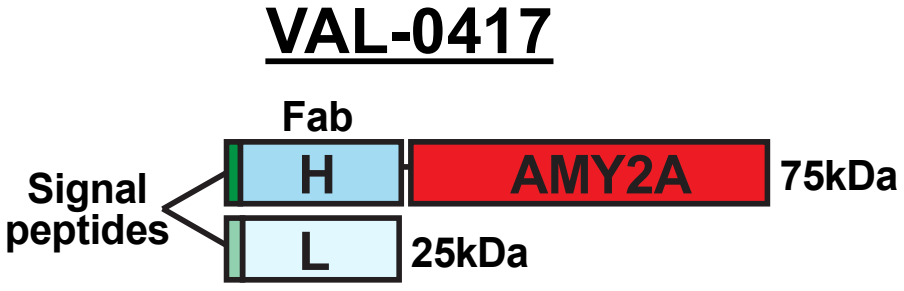
Antibody-enzyme fusion (AEF) treatment platform

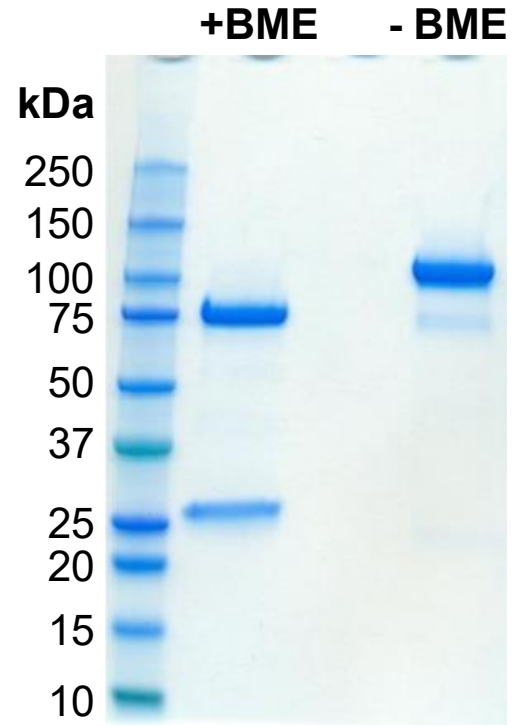


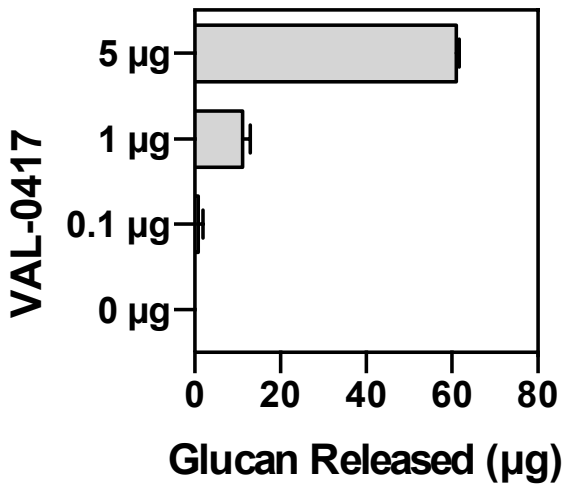


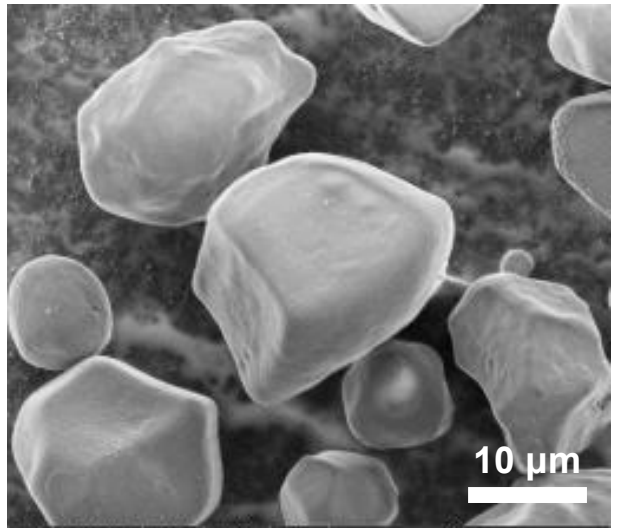
Weisbart et al., 1990 *J Immunology*
Hansen et al., 2007 *JBC*
Hansen et al., 2012 *Sci Trans Med*
Yi et al., 2017 *Human Mol Gen*

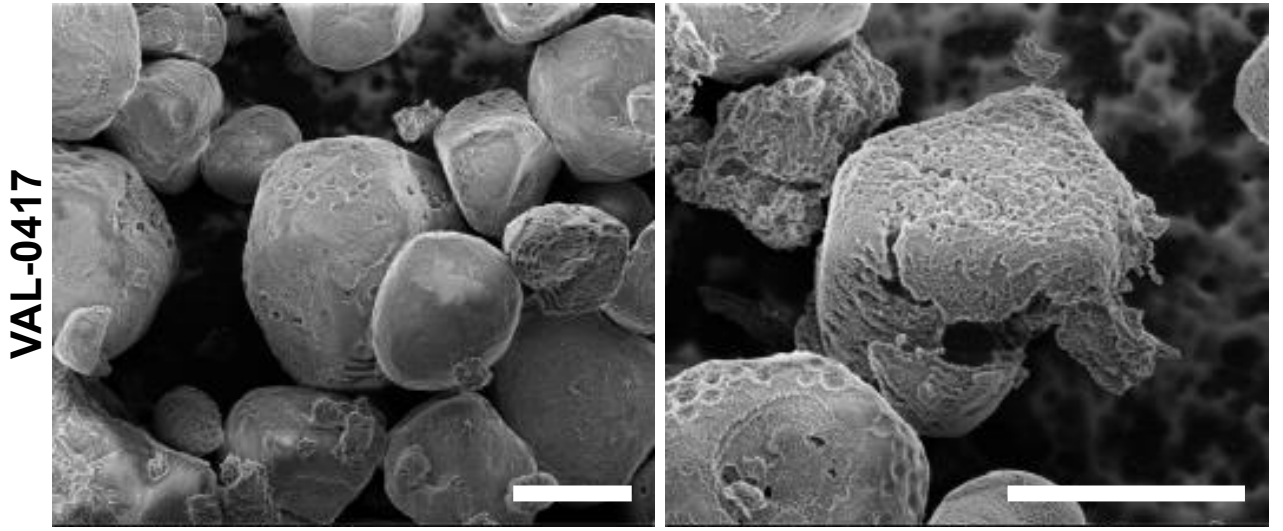
The AEF VAL-0417 degrades corn starch





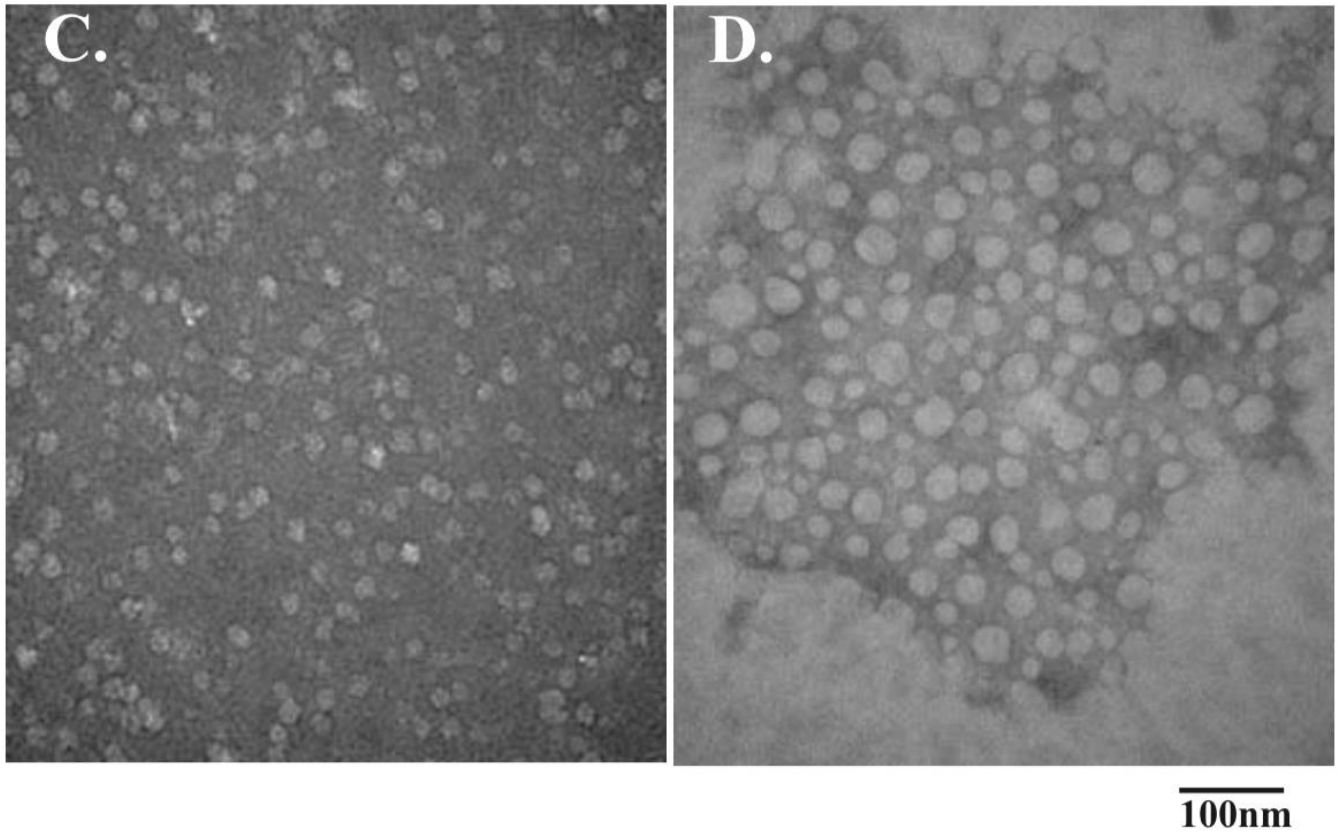






Denaturing vs. Native Methods for LB purification

9-12 month old WT **9-12 month old *Epm2a-/-***

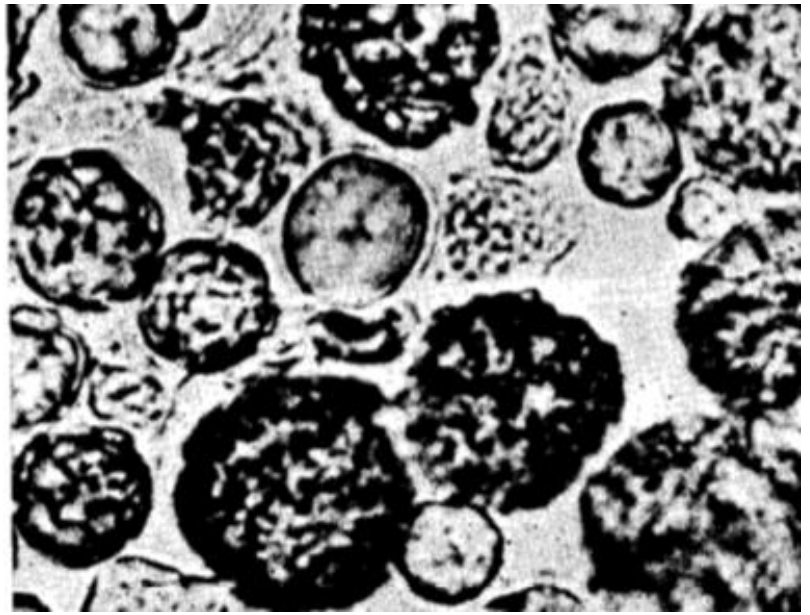


Tagliabracci, et al. *JBC* (2008).

Studies in Myoclonus Epilepsy
(Lafora Body Form)

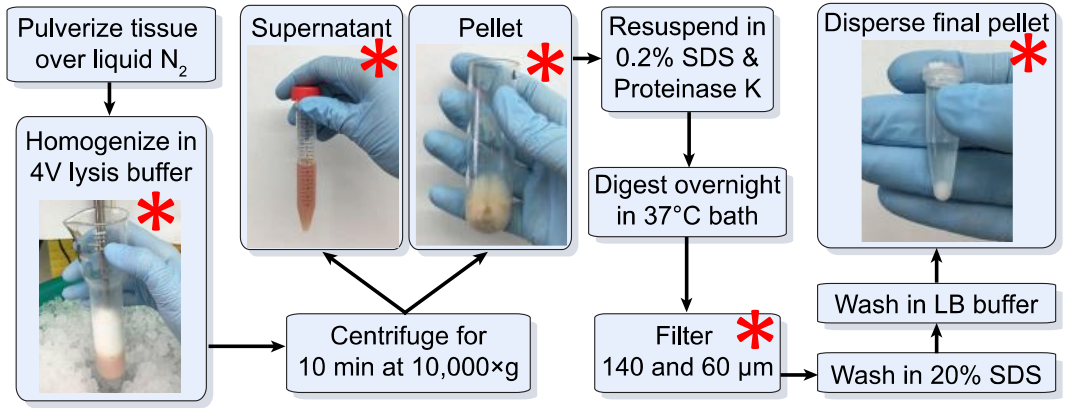
I. Isolation and Preliminary
Characterization of Lafora Bodies in Two Cases

Susumu Yokoi, MD; James Austin, MD;
Frank Witmer, ScD; and Masao Sakai, MD, Denver



Yokoi et al. *Arch Neurol.*1968.

Purification of native LBs from *Epm2a-/-* and *Epm2b-/-* mice



* Samples removed for yield quantitation

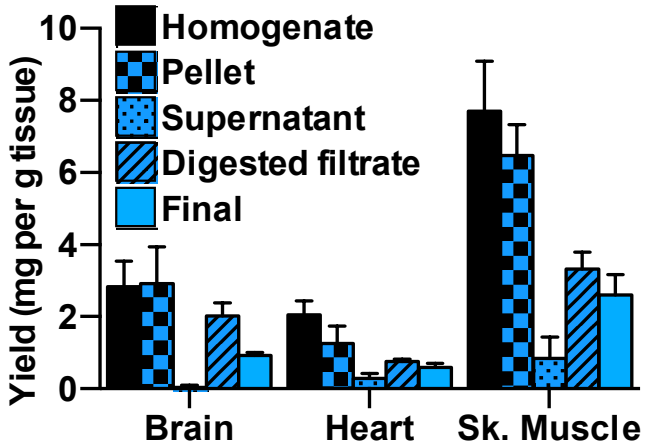
↓

Boiled in 30% KOH
Precipitated with ethanol + LiCl
Resuspended in water

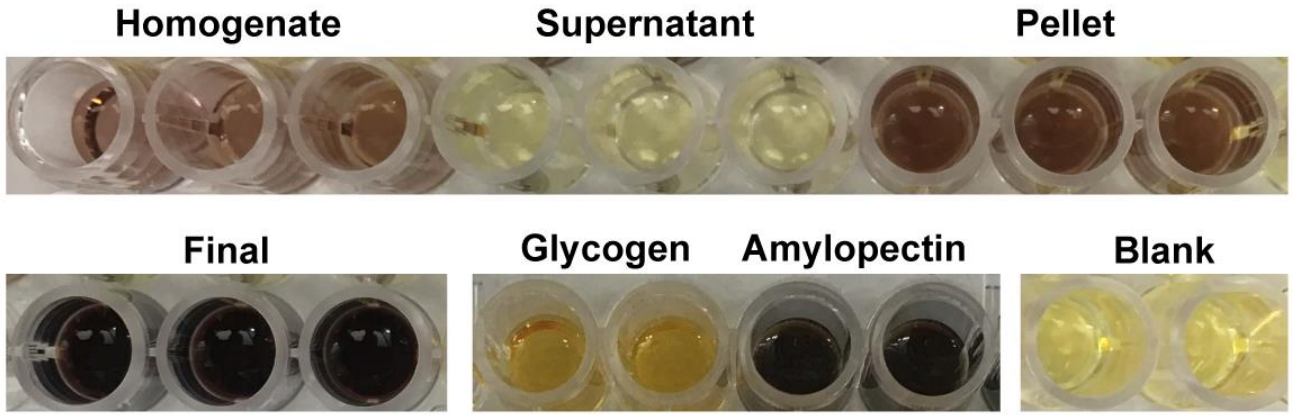
↓

Digested with amyloglucosidase
Total glucose quantified by G6PDH/HK

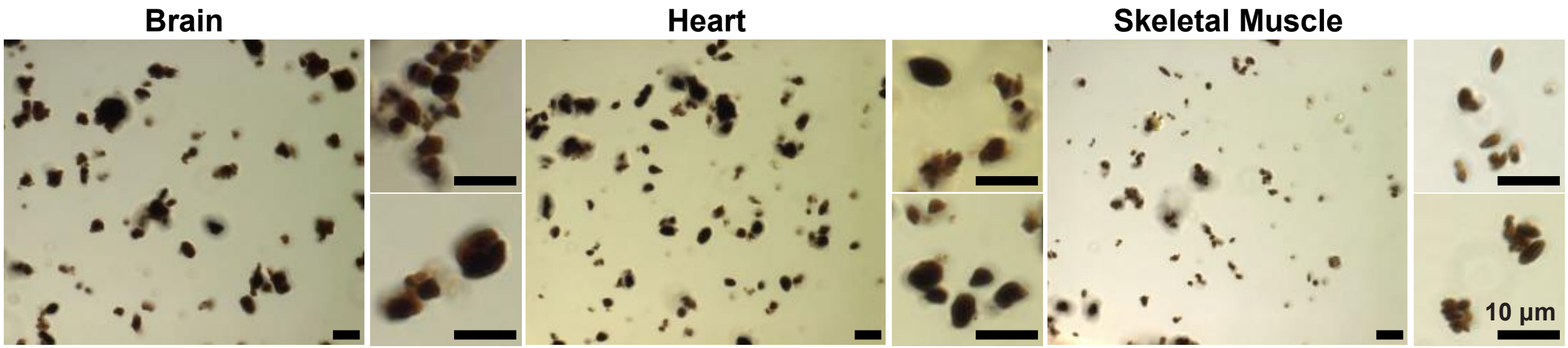
	Tissue weight (g)	Homogenate		Pellet		Supernatant		Digested filtrate		Final	
		yield (mg)	% of total	yield (mg)	% of total	yield (mg)	% of total	yield (mg)	% of total	yield (mg)	% of total
Brain	3.06	8.63	100.00	8.97	103.91	0.14	1.58	6.21	71.92	2.84	32.94
Heart	1.64	3.35	100.00	2.07	61.71	0.48	14.32	1.24	36.99	0.98	29.37
Sk. M.	3.35	25.81	100.00	21.71	84.14	2.80	10.86	11.16	43.25	8.77	33.99

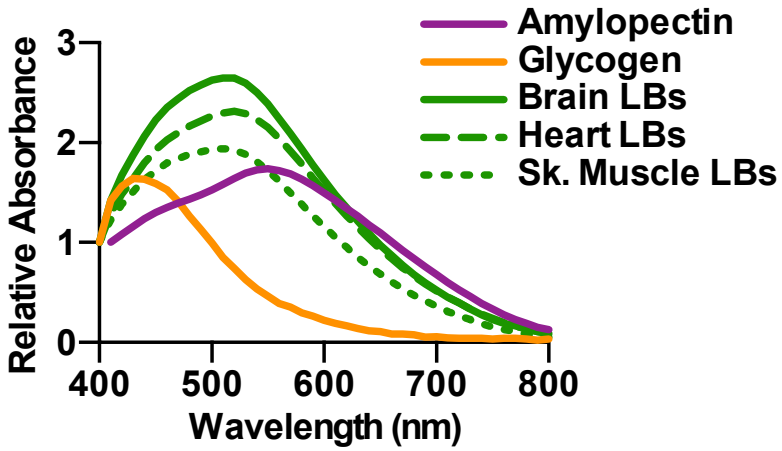


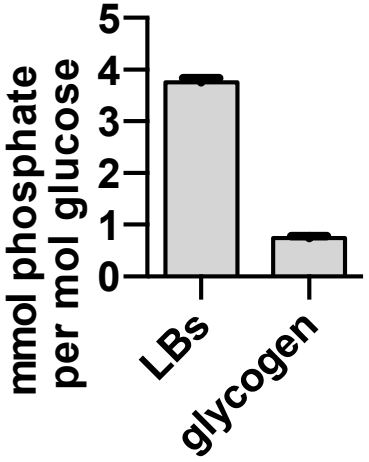
Polysaccharide = glycogen + polyglucosan (denatured LBs)



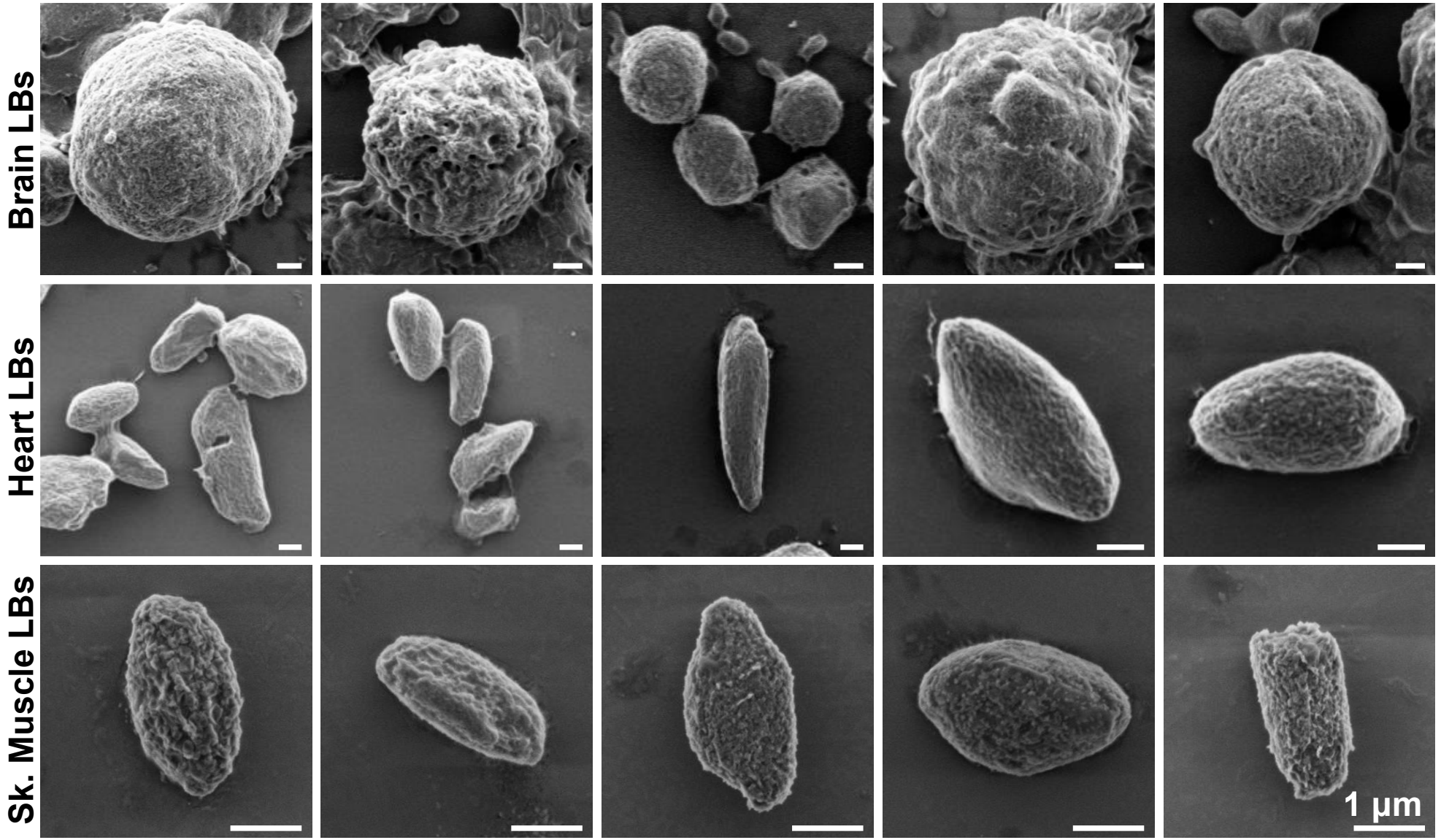
Purification of native LBs from *Epm2a-/-* and *Epm2b-/-* mice



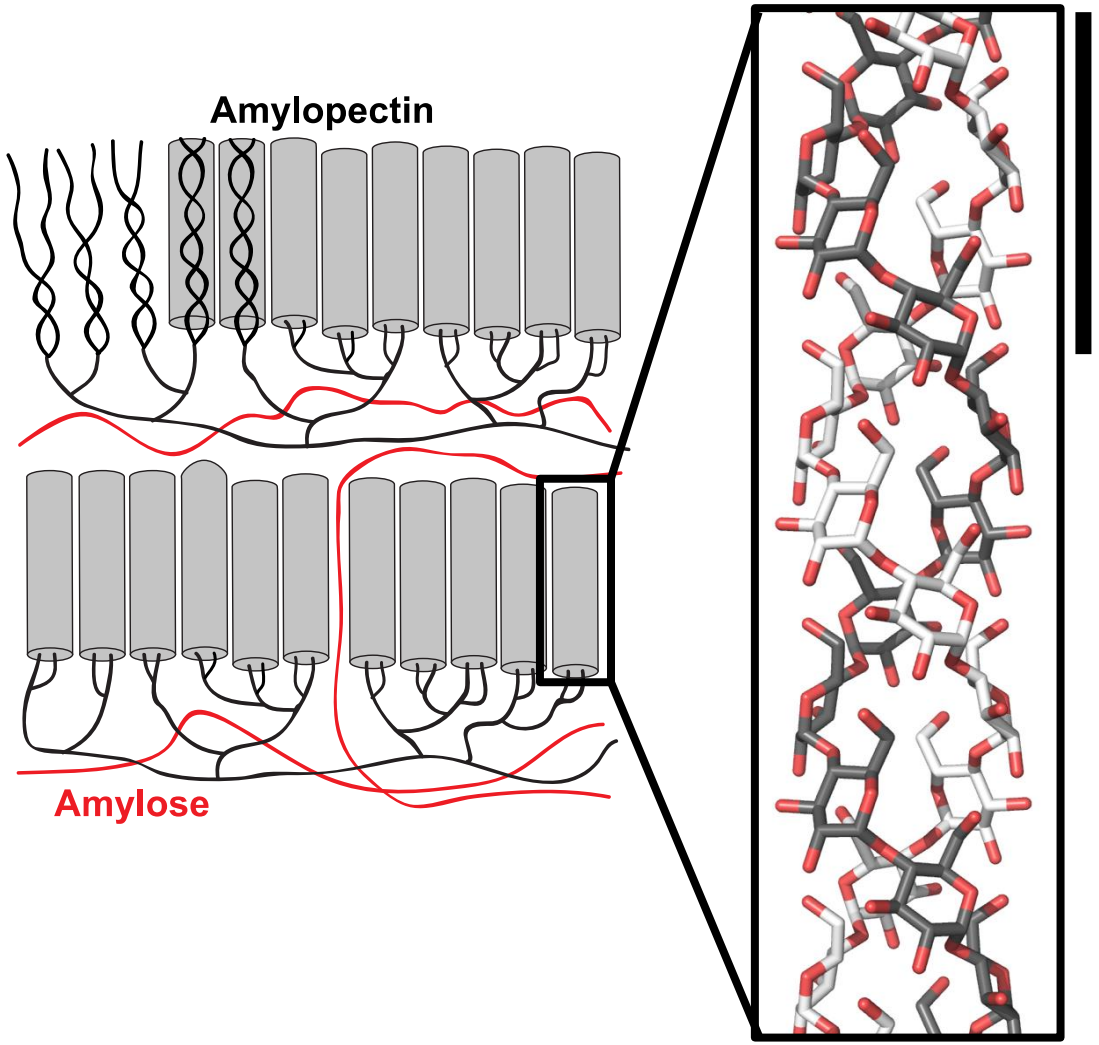




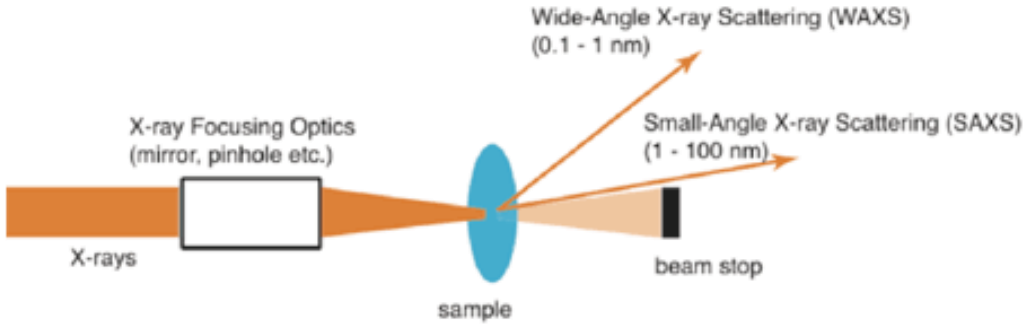
Purification of native LBs from *Epm2a-/-* and *Epm2b-/-* mice



Crystallinity in LBs

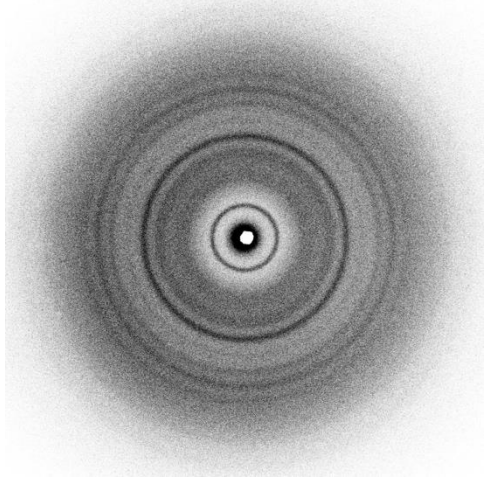


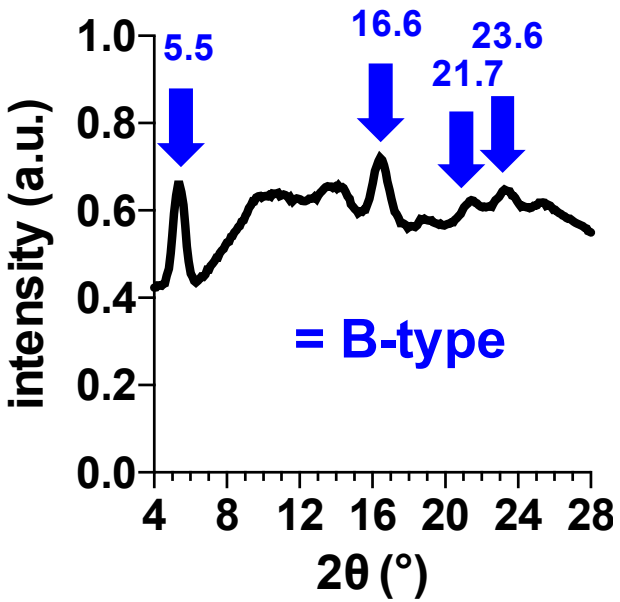
Wide-angle X-ray scattering (WAXS)



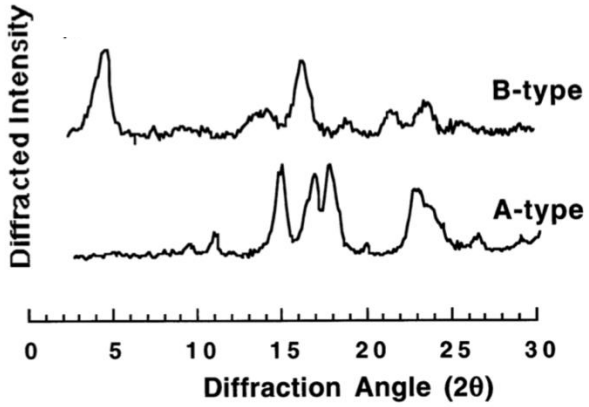
Crystallinity in LBs

Sk. Muscle LBs

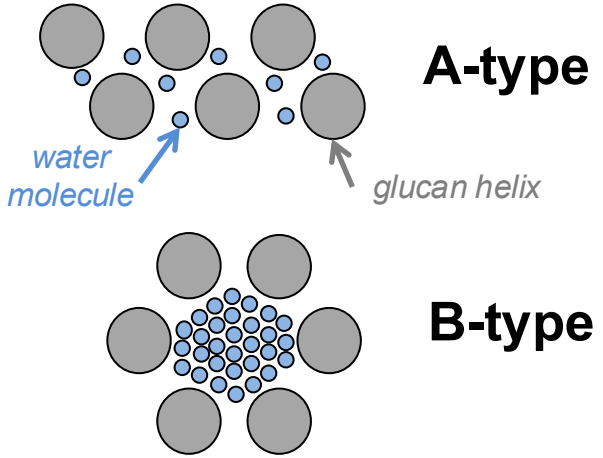




WAXS reflection angles indicate crystallinity type



Buleon et al., *Int J Biol Macromol* (1998).



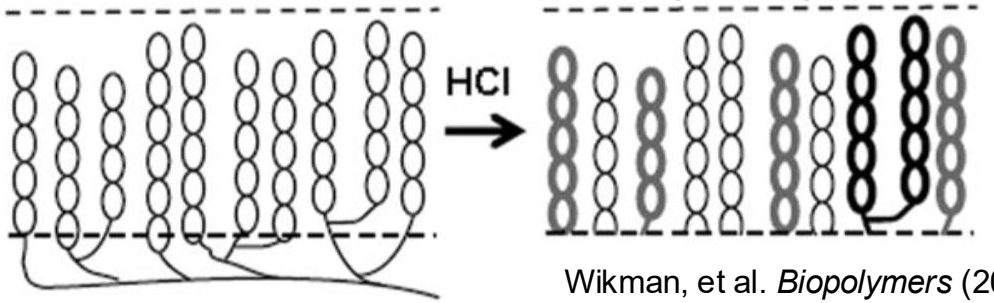
Adapted from Qiao, et al. *Int J Biol Macromol* (2017).

Jean-Luc Putaux
Plant Macromolecules Research Center (CERMAV)
University of Grenoble, France



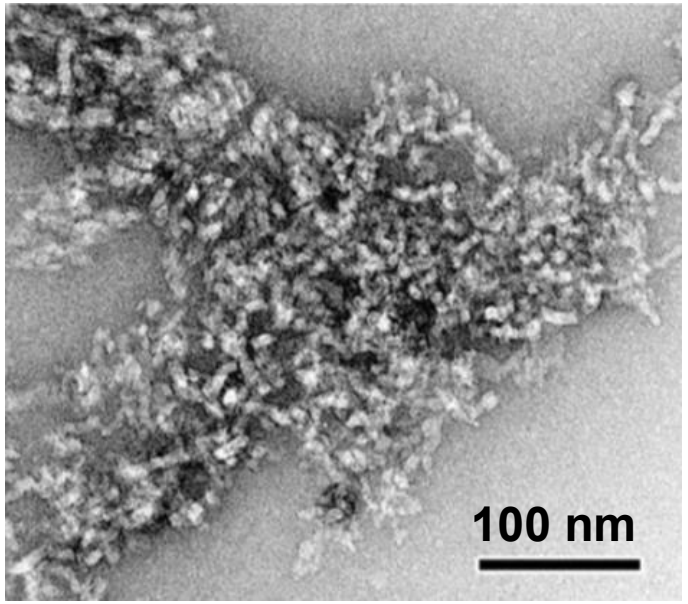
Crystallinity in LBs

Lintnerization = mild acid hydrolysis



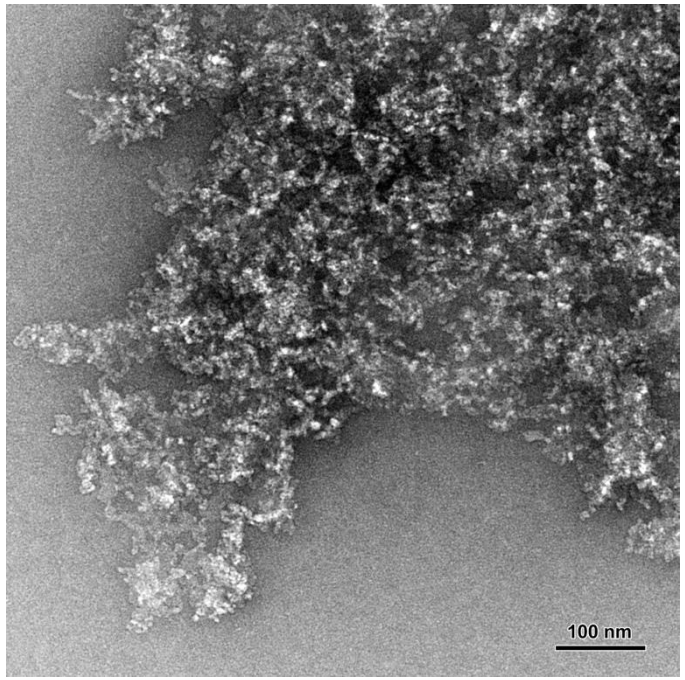
Wikman, et al. *Biopolymers* (2014).

lintnerized barley starch
2.2 N HCl for 35 days

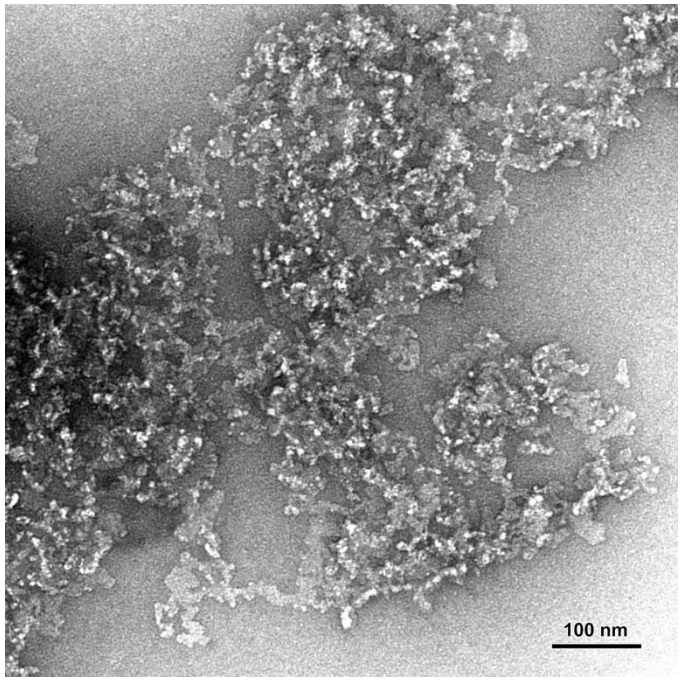


Goldstein, et al. *Int J Biol Macromol* (2016).

Lintnerized Brain LBs



Lintnerized Sk. Muscle LBs



2.2 N HCl
5 days

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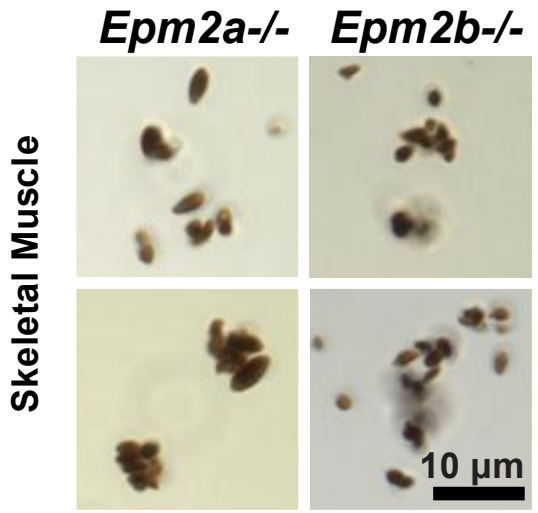
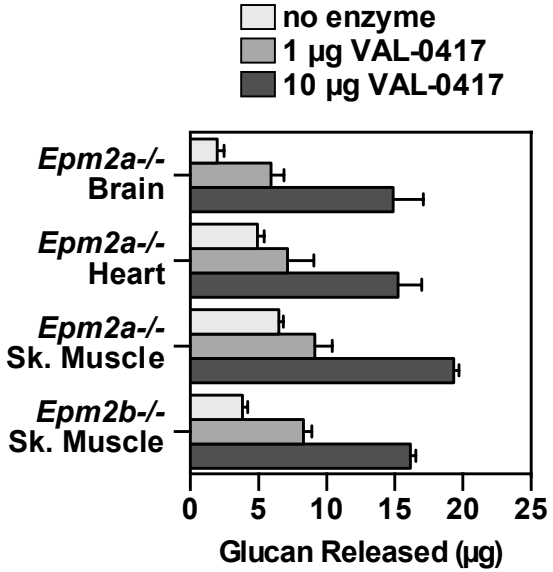
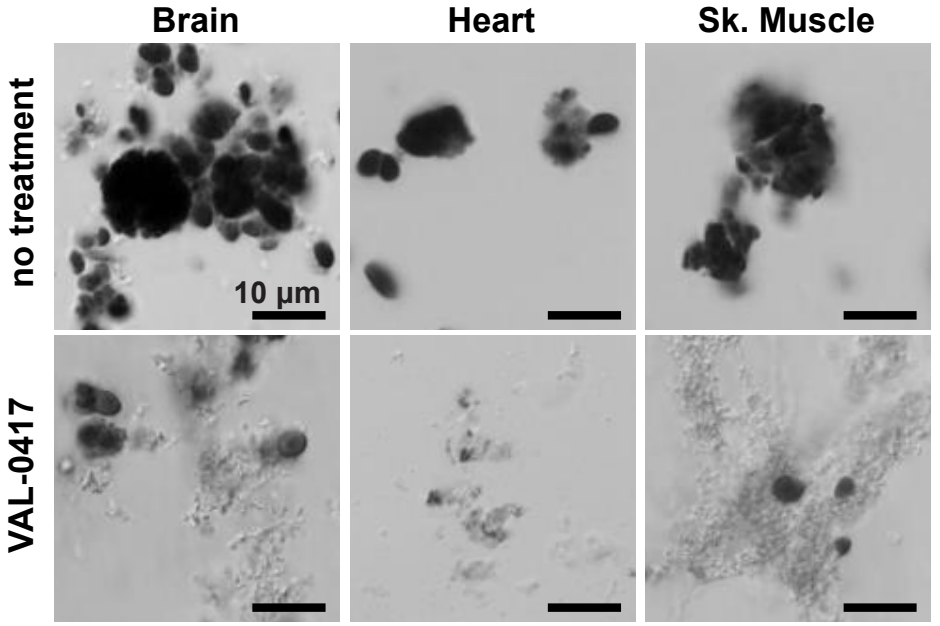
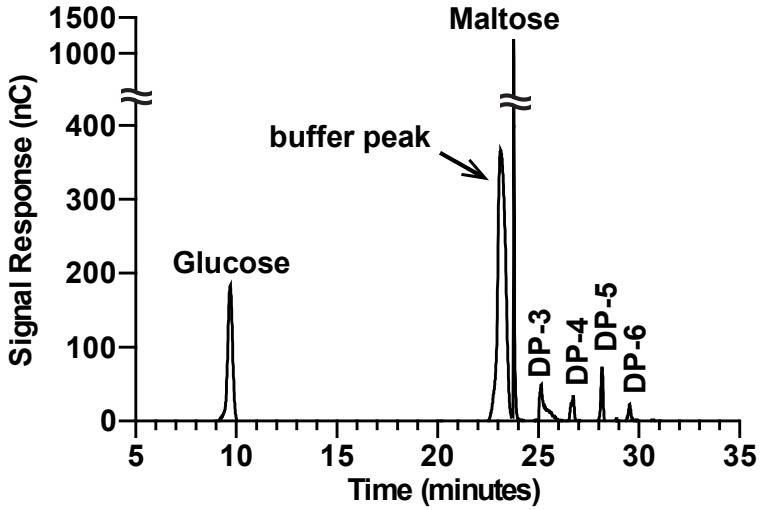
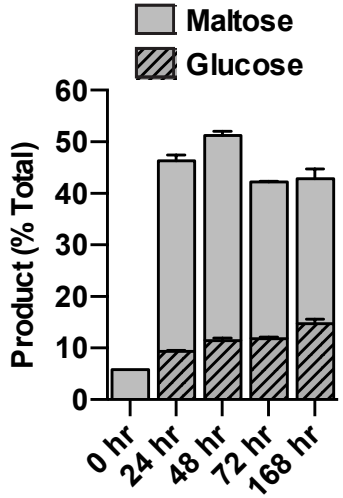
VAL-0417 degrades LBs *in vitro*



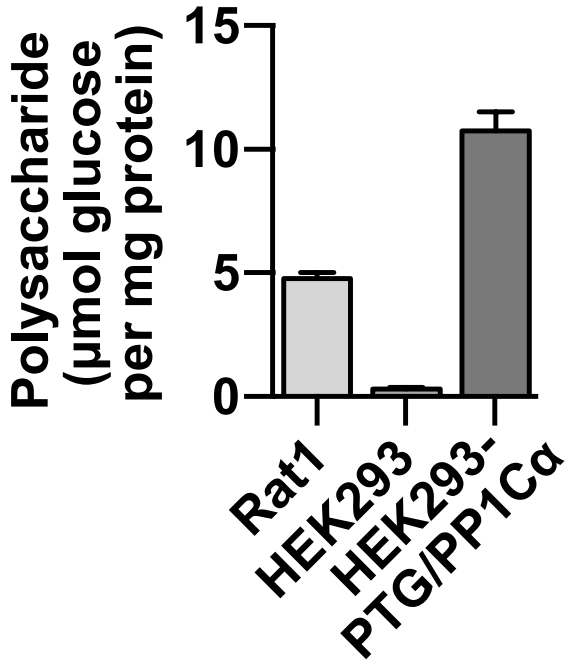


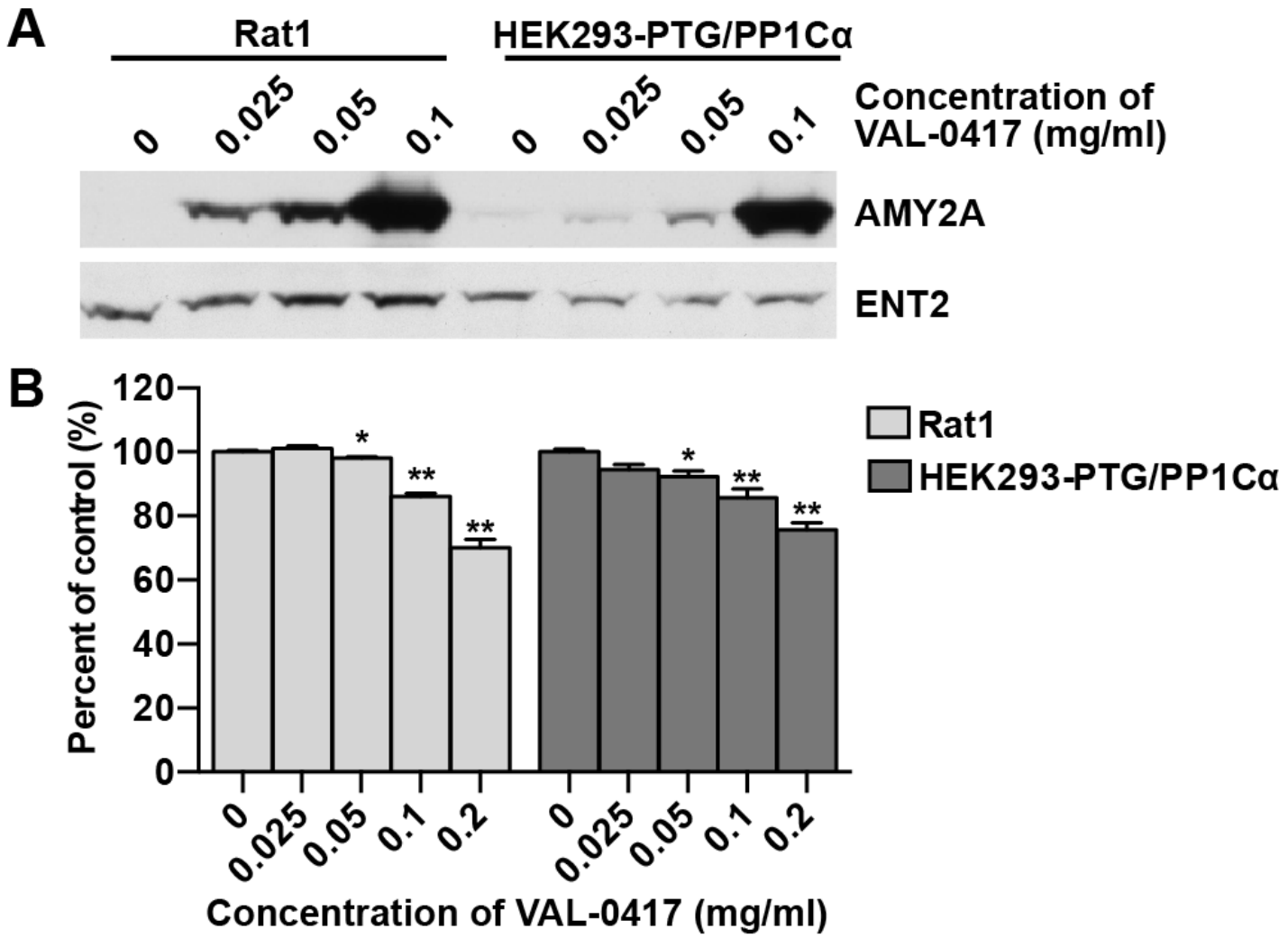






VAL-0417 uptake and polysaccharide degradation

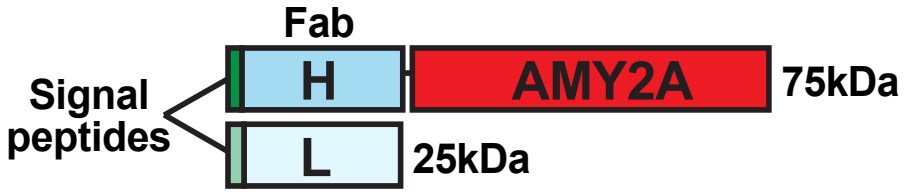




Dyann Segich, Anna DePaoli-Roach & Peter Roach

A sandwich ELISA to detect VAL-0417 levels

VAL-0417

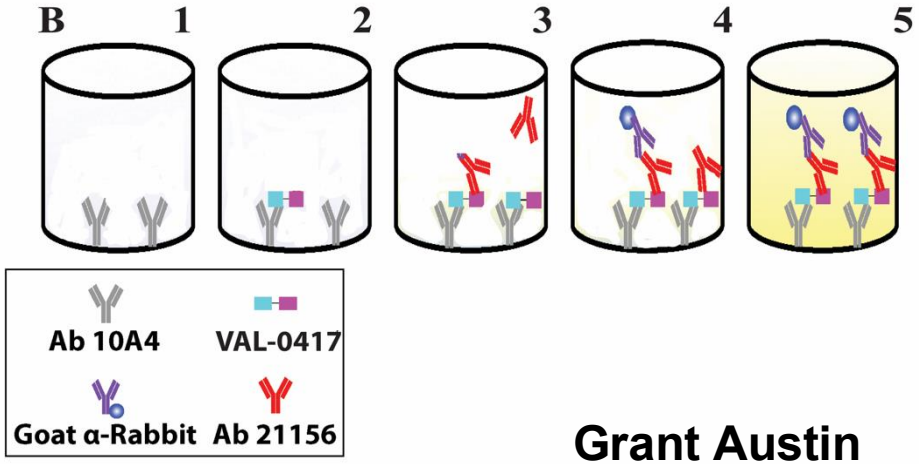


Intramuscular injection

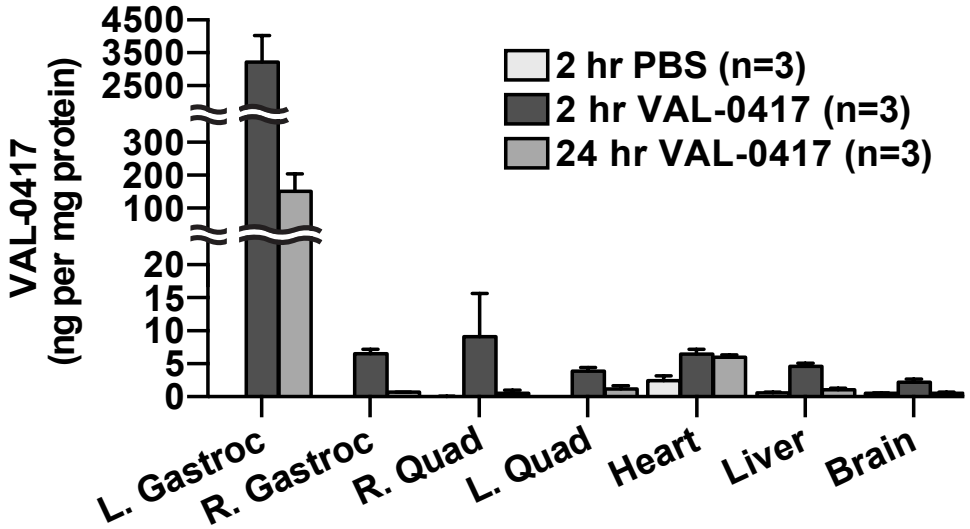


C57Bl6 WT mice

Injected 0.6 mg VAL-0417 or PBS in left gastroc → Euthanized 2 and 24 hours post-injection Multiple tissues collected → VAL-0417 levels determined by ELISA



Grant Austin



VAL-0417 degrades skeletal muscle LBs *in vivo*

Intramuscular injections



10 month old WT and *Epm2a-/-* mice

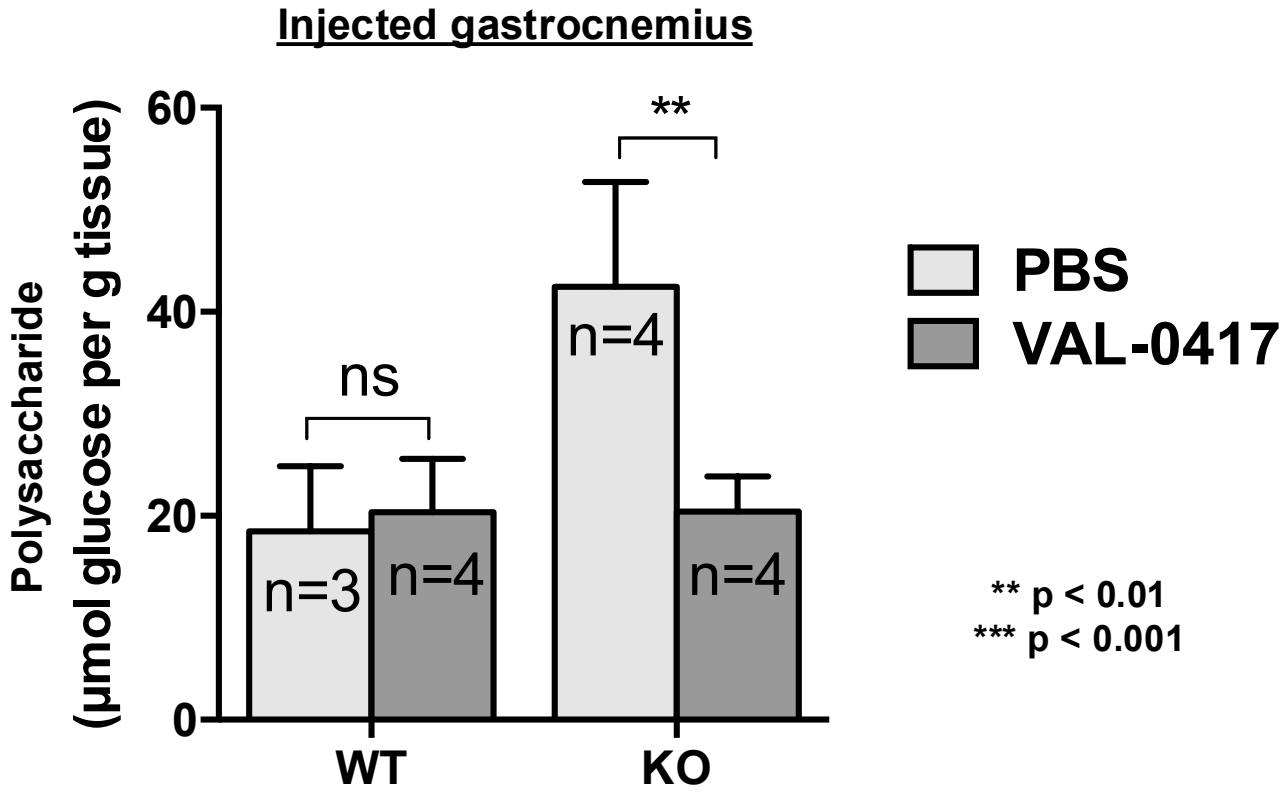
3 injections of 0.6 mg VAL-0417 or PBS in right gastroc over 7 days

↓

Euthanized on 8th day Gastrocs collected

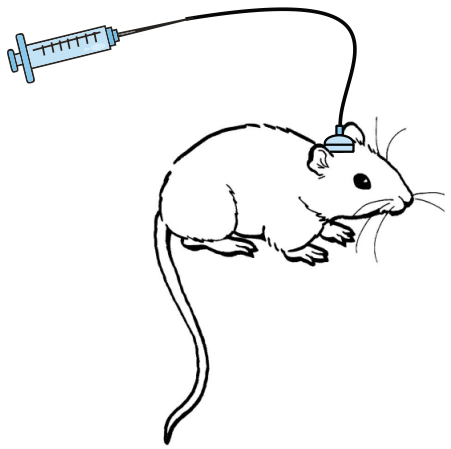
↓

Polysaccharide content determined



VAL-0417 reduces cerebral LB load *in vivo*

Intracerebroventricular (ICV) administration

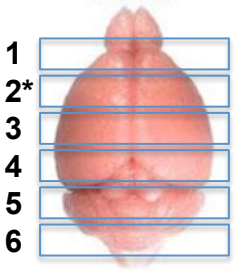


5 month old
Epm2a-/- mice

Continuous infusion of VAL-0417 (0.08mg/day) or PBS for 28 days via ICV catheter

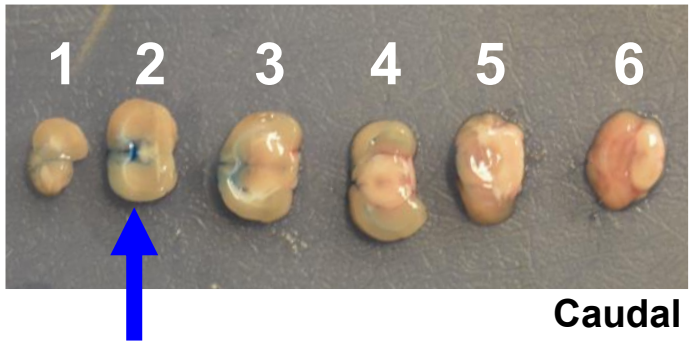
NBR
Northern Biomedical Research, Inc.

Euthanized on 29th day
Brains sectioned



Polysaccharide content determined

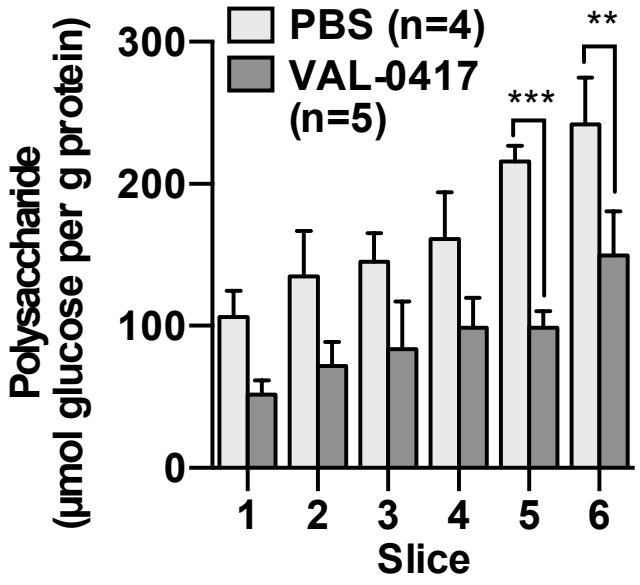
Rostral

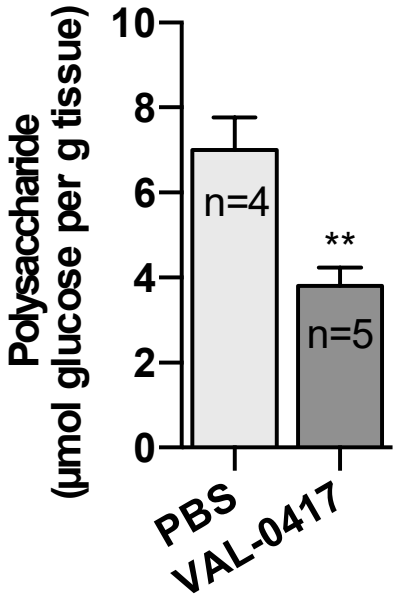


Caudal

Blue dye injected via ICV catheter

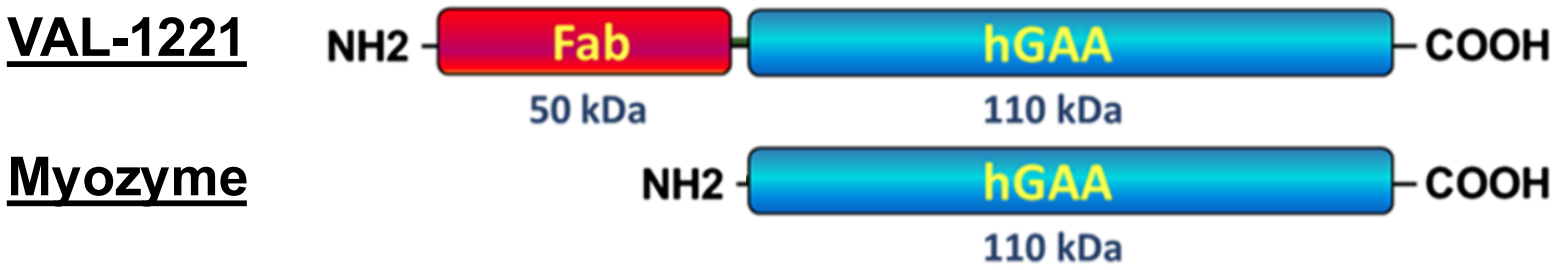
Whole brain





** p < 0.01
*** p < 0.001

VAL-1221 vs. Myozyme (rhGAA) in Pompe mice



VAL-1221 Delivered Intravenously in Ambulatory and Ventilator-free Patients With Late-Onset Pompe Disease

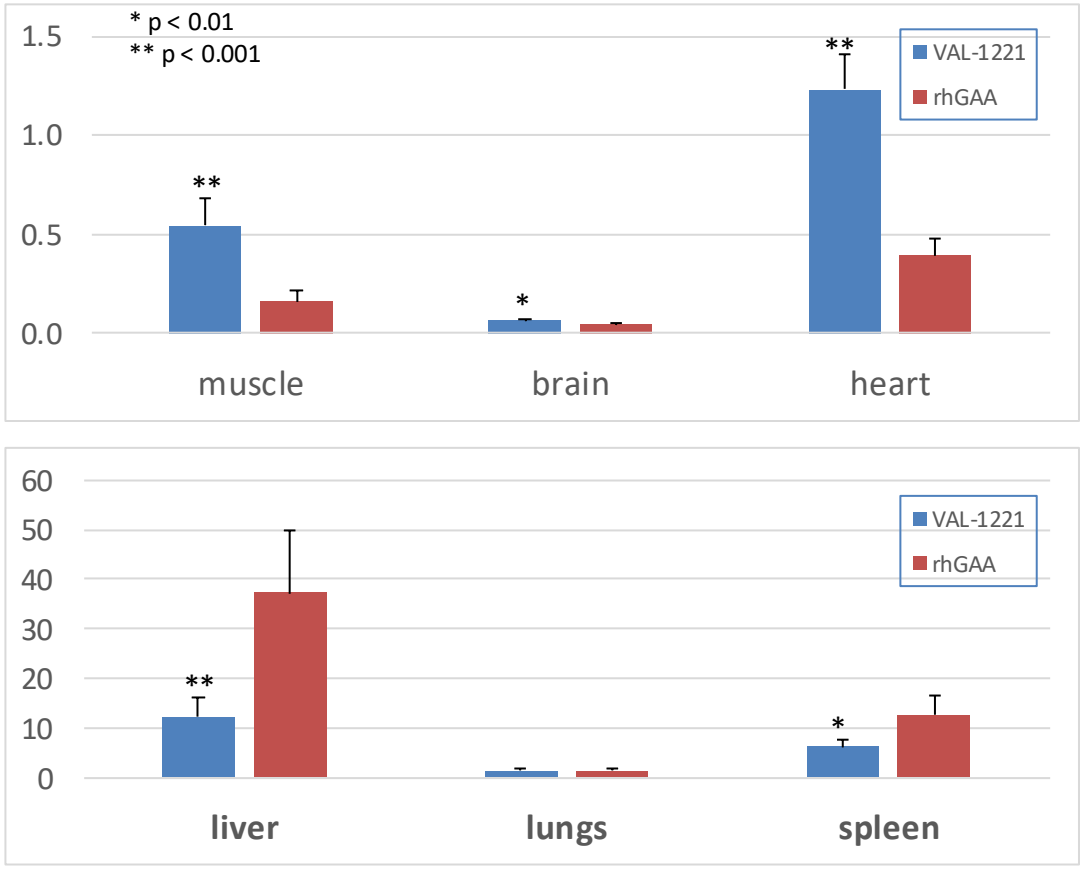
ClinicalTrials.gov Identifier: NCT02898753

VAL-1221 vs. Myozyme (rhGAA) in Pompe mice

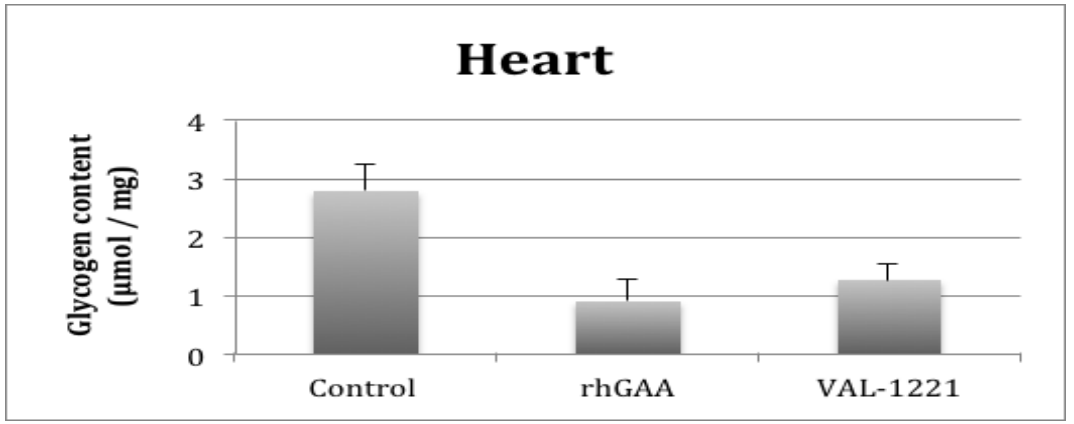
VAL-1221

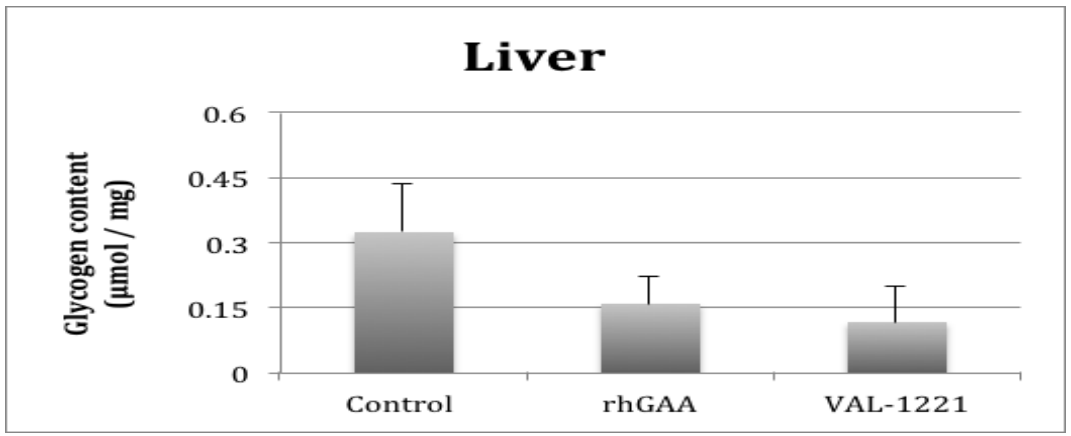


Improved Biodistribution



Equivalent Glycogen Clearance





VAL-1221 reduces skeletal muscle LBs *in vivo*

Tail vein (IV) injections



9 month old
WT and *Epm2a-/-* mice

4 injections of 0.9 mg
VAL-1221 in tail vein
over 14 days

↓

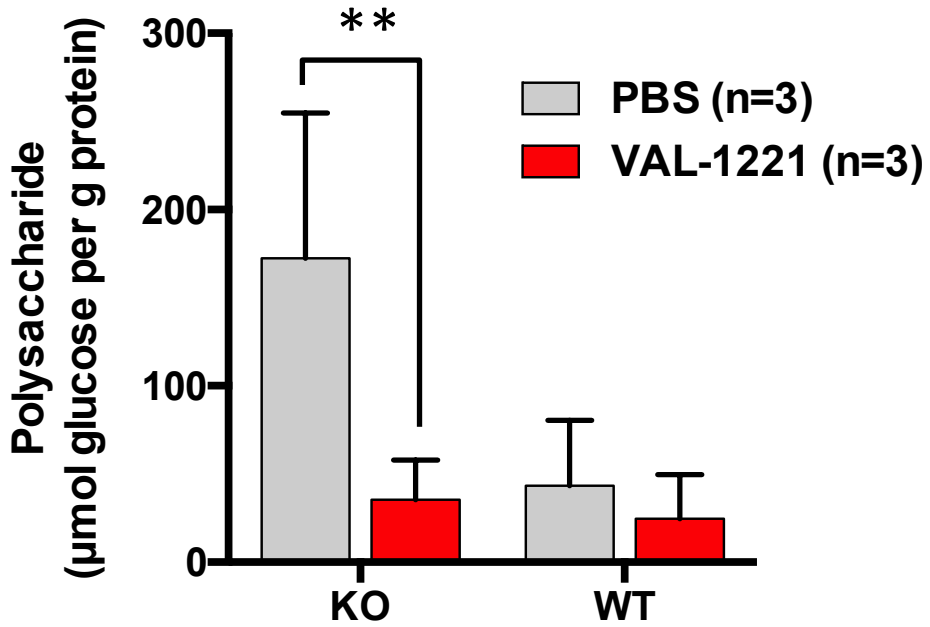
Euthanized on 18th day
Multiple tissues
collected

↙ ↘

Polysaccharide content determined

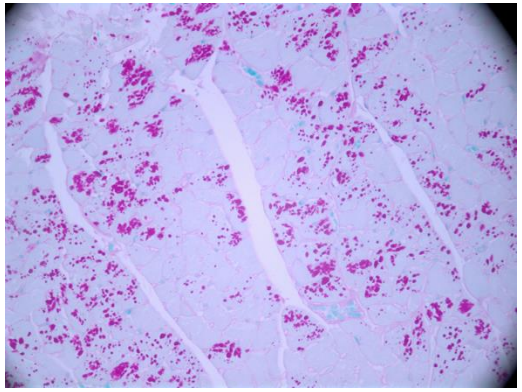
PAS staining

Quadriceps



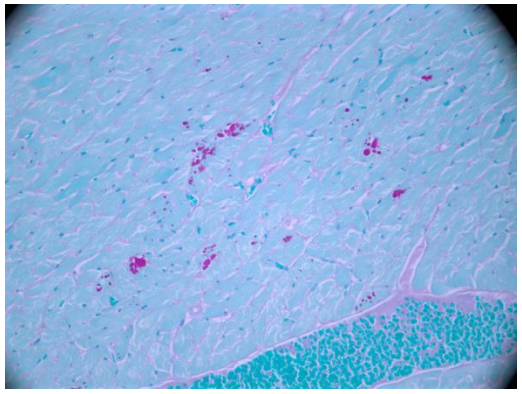
** p < 0.01

PBS treated (gastroc)



>70% PAS Positive Fibers

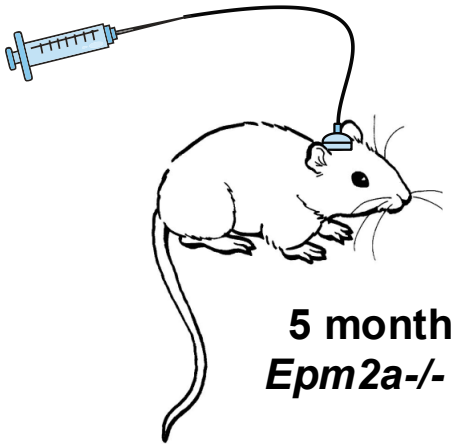
VAL-1221 treated (gastroc)



~10% PAS Positive Fibers

VAL-1221 reduces cerebral LB load as well as VAL-0417

Intracerebroventricular (ICV) administration

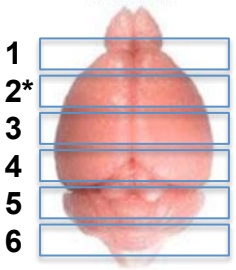


5 month old *Epm2a-/-* mice

Continuous infusion of VAL-1221 (0.08mg/day) for 28 days via ICV catheter

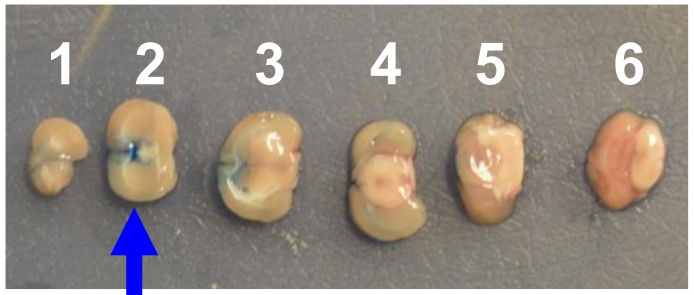
NBR Northern Biomedical Research, Inc.

→ Euthanized on 29th day Brains sectioned →



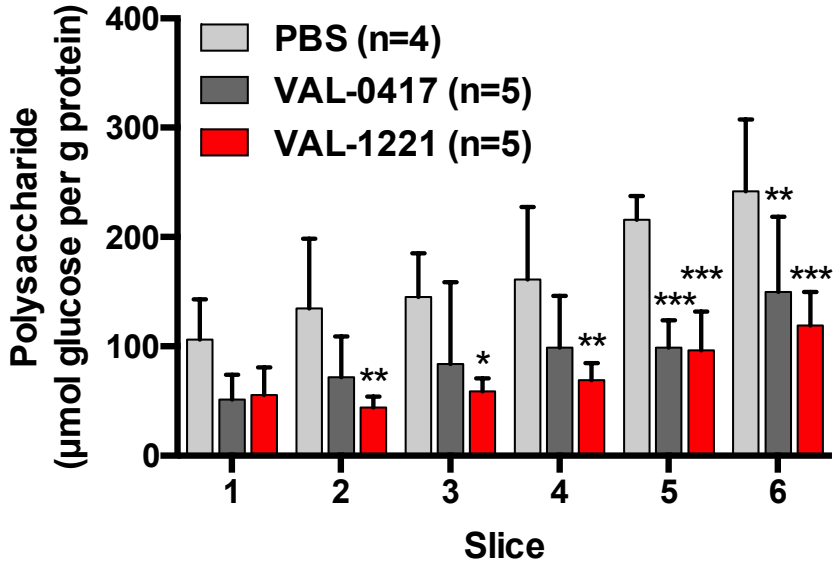
→ Polysaccharide content determined

Rostral

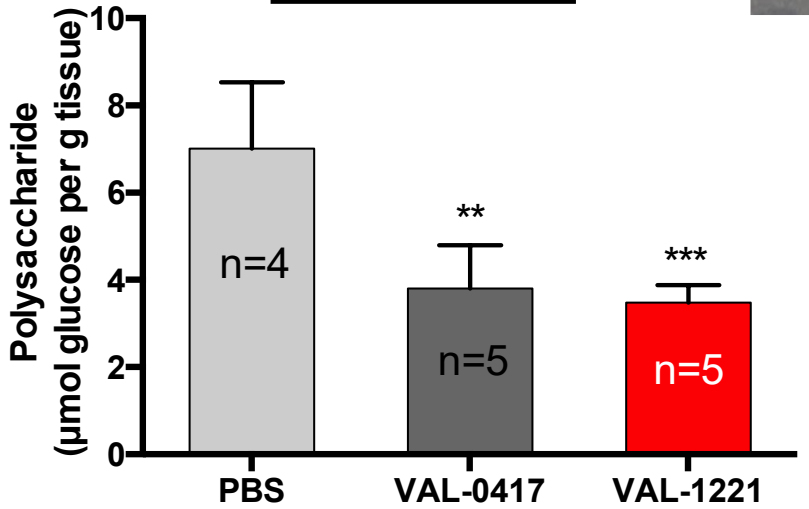


Caudal

Blue dye injected via ICV catheter



Whole brain



** p < 0.01
*** p < 0.001

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