Cold Spring Harbor Perspectives in Biology (2012)

Issue: Extracellular Matrix

Cold Spring Harb Perspect Biol 2012

Overview of the "Matrisome"—An Inventory of Extracellular Matrix Constituents and Functions

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- Completion of genome sequences for many organisms defines the complete "list" of extracellular matrix (ECM) proteins.
- In mammals: **"core matrisome" comprises ~300 proteins**. Also: large numbers of ECM modifying *enzymes* & ECM-binding *growth factors*
- These ECM & ECM-associated proteins cooperate to assemble & remodel extracellular matrices and bind to cells through receptors so cells can survive, proliferate, differentiate, shape, and migrate.
- **ECM proteins were the key to the transition to multi-cellularity, the *arrangement of cells into tissues*, and the elaboration of novel structures during vertebrate evolution.**

PROTEOGLYCAN SUPERFAMILY

- **ECM molecules with (1) Core protein, and (2) Glycosaminoglycan (GAG) chains**

- **“Sub-families” include**

  - Extracellular
    - **Large Aggregating (Aggrecan)**
    - Small Leucine-Rich PG (SLRPs)
  - Cell Surface (e.g., glycocalyx HSPGs)

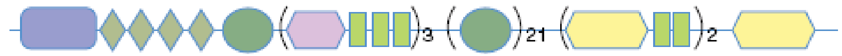
Table 1. Extracellular matrix proteoglycans (Proteoglycan Superfamily)

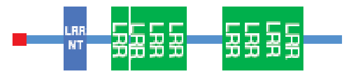
(36 - 42 or more)

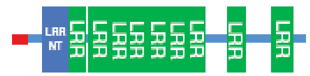
SLRPS

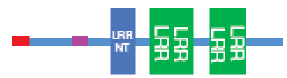
Large Aggregating PGs

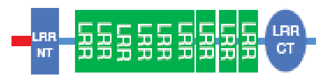
GENE NAME	COMMON NAME(S)	DOMAINS	GAGs
HSPG2	heparan sulfate proteoglycan 2/**perlecan**	complex	HS/CS
ASPN	asporin	LRR	maybe none
BGN	**biglycan**	LRR	CS/DS
DCN	decorin	LRR	CS/DS
FMOD	fibromodulin	LRR	KS
KERA	keratocan	LRR	KS
LUM	**lumican**	LRR	KS
OMD	osteomodulin/osteoadherin	LRR	KS
PRELP	PRELP/prolargin (pro/arg-end/leu-rich repeat protein)	LRR	KS ??
EPYC	**epiphycan**	LRR	CS/DS
OGN	osteoglycin/mimecan	LRR	KS
OPTC	opticin	LRR	??
CHAD	**chondroadherin**	LRR	maybe none
CHADL	chondroadherin-like	LRR	maybe none
NYX	nyctalopin (probably GPI-linked)	LRR	maybe none
NEPNP	nephrocan (pseudogene in human)	LRR	
PODN	**podocan**	LRR	maybe none
PODNL1	podocan-like 1	LRR	maybe none
ACAN	aggrecan	LINK/CLEC/CCP	CS/KS
BCAN	**brevican**	LINK/CLEC/CCP	CS
NCAN	neurocan	LINK/CLEC/CCP	CS
VCAN	versican	LINK/CLEC/CCP	CS/DS
HAPLN1	**hyaluronan and proteoglycan link protein 1**	LINK	
HAPLN2	hyaluronan and proteoglycan link protein 2	LINK	
HAPLN3	hyaluronan and proteoglycan link protein 3	LINK	
HAPLN4	hyaluronan and proteoglycan link protein 4	LINK	
PRG2	proteoglycan 2, bone marrow PG	CLEC	
PRG3	**proteoglycan 3**	CLEC	
SPOCK1	testican 1	SPARC, Kazal, TY	CS/KS
SPOCK2	testican 2	SPARC, Kazal, TY	CS/KS
SPOCK3	testican 3	SPARC, Kazal, TY	CS/KS
PRG4	proteoglycan 4/lubricin	SO/HX	maybe none
SRGN	serglycin	serglycin	HS/CS
IMPG1	interphotoreceptor matrix proteoglycan 1	SEA domain	CS
IMPG2	interphotoreceptor matrix proteoglycan 2	SEA domain	CS
ESM1	endocan/endothelial cell-specific molecule 1	IB domain	CS/DS

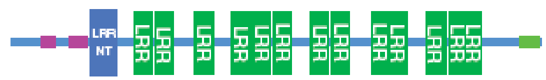


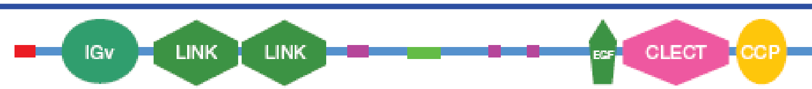


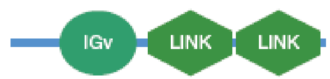


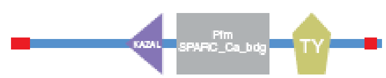






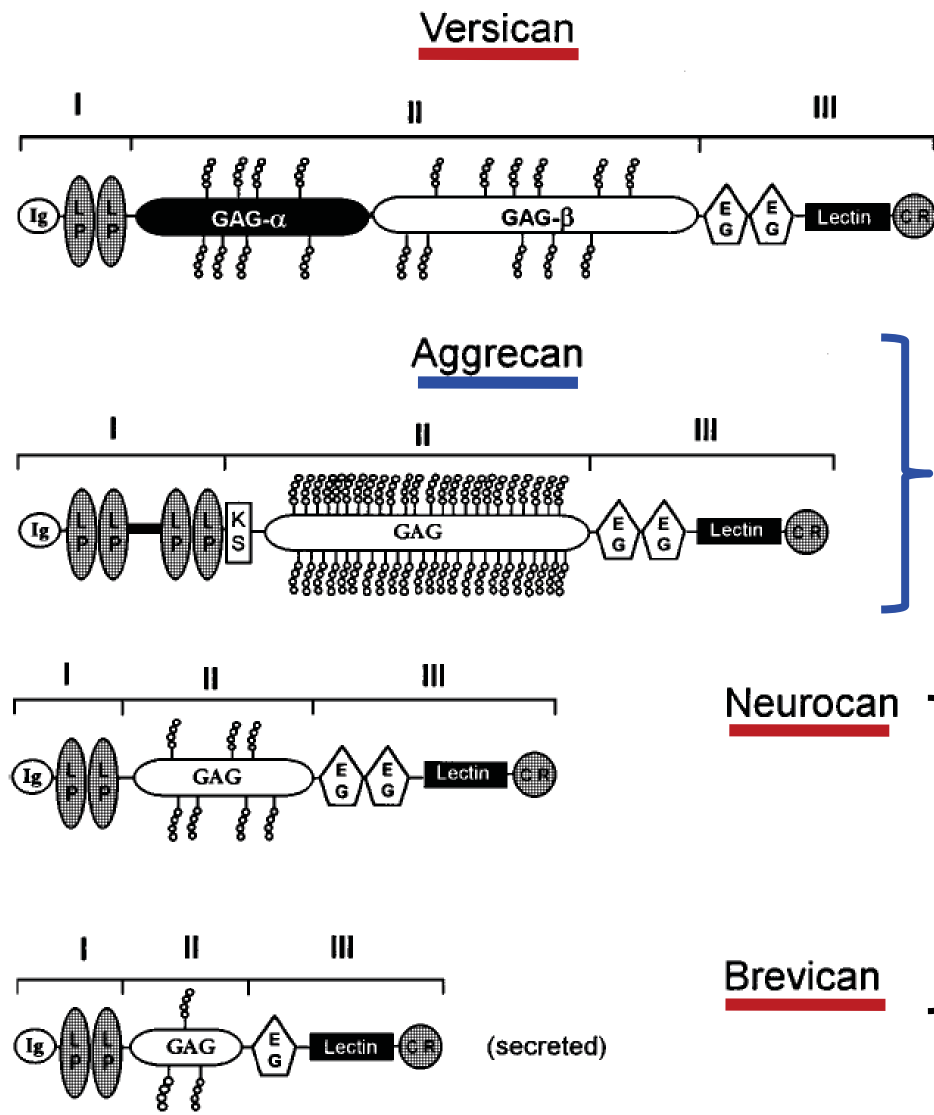






Source: Hynes, Richard O., and Alexandra Naba. "Overview of the Matrisome—An Inventory of Extracellular Matrix Constituents and Functions." *Cold Spring Harbor Perspectives in Biology* 4, no. 1 (2012): a004903.

Aggrecan: a member of "Subfamily" of aggregating proteoglycans ("hyalectans")



blood vessel walls

Great in Compression
(disc, cartilage, tendons)

In brain & other nerve tissue

Large Aggregating Proteoglycans in Brain

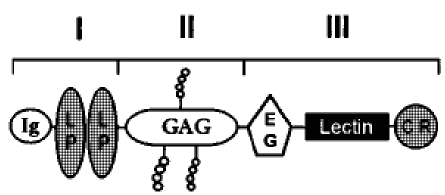
Physiological Reviews 2000

Proteoglycans in the Developing Brain: New Conceptual Insights for Old Proteins

CHRISTINE E. BANDTLOW AND DIETER R. ZIMMERMANN

Brain Research Institute, University of Zurich and Swiss Federal Institute of Technology Zurich; and Molecular Biology Laboratory, Department of Pathology, University Hospital, Zurich, Switzerland

Proteoglycans, a group of glycoproteins that carry covalently bound sulfated glycosaminoglycan (GAG) chains, are molecules that have "come of age." Recognized in the early 1960s as important structural components of the extracellular matrix of cartilage, proteoglycans were once thought to be specific to that tissue. By now it has become clear that they are found in the matrices of all tissues, including the brain.

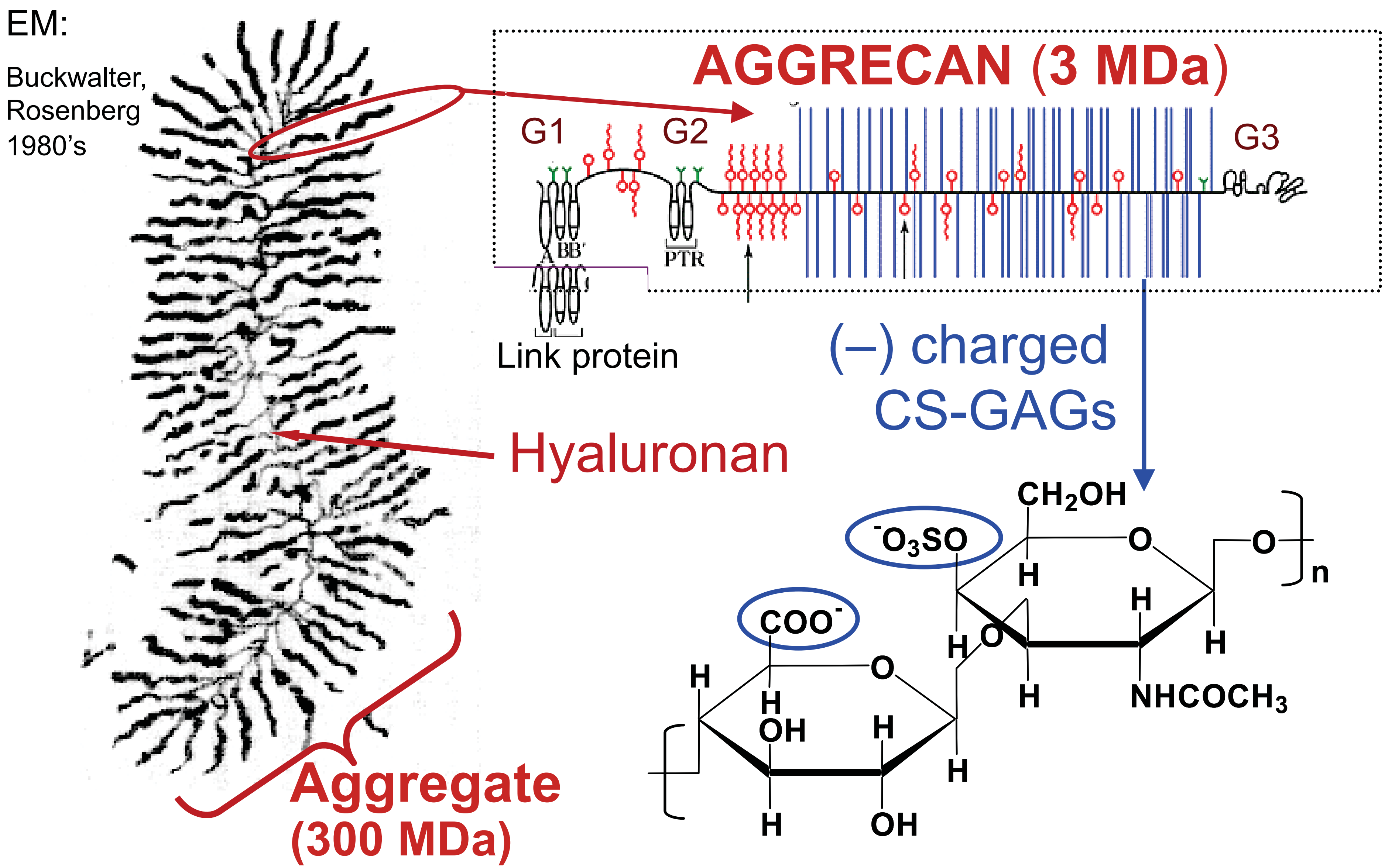


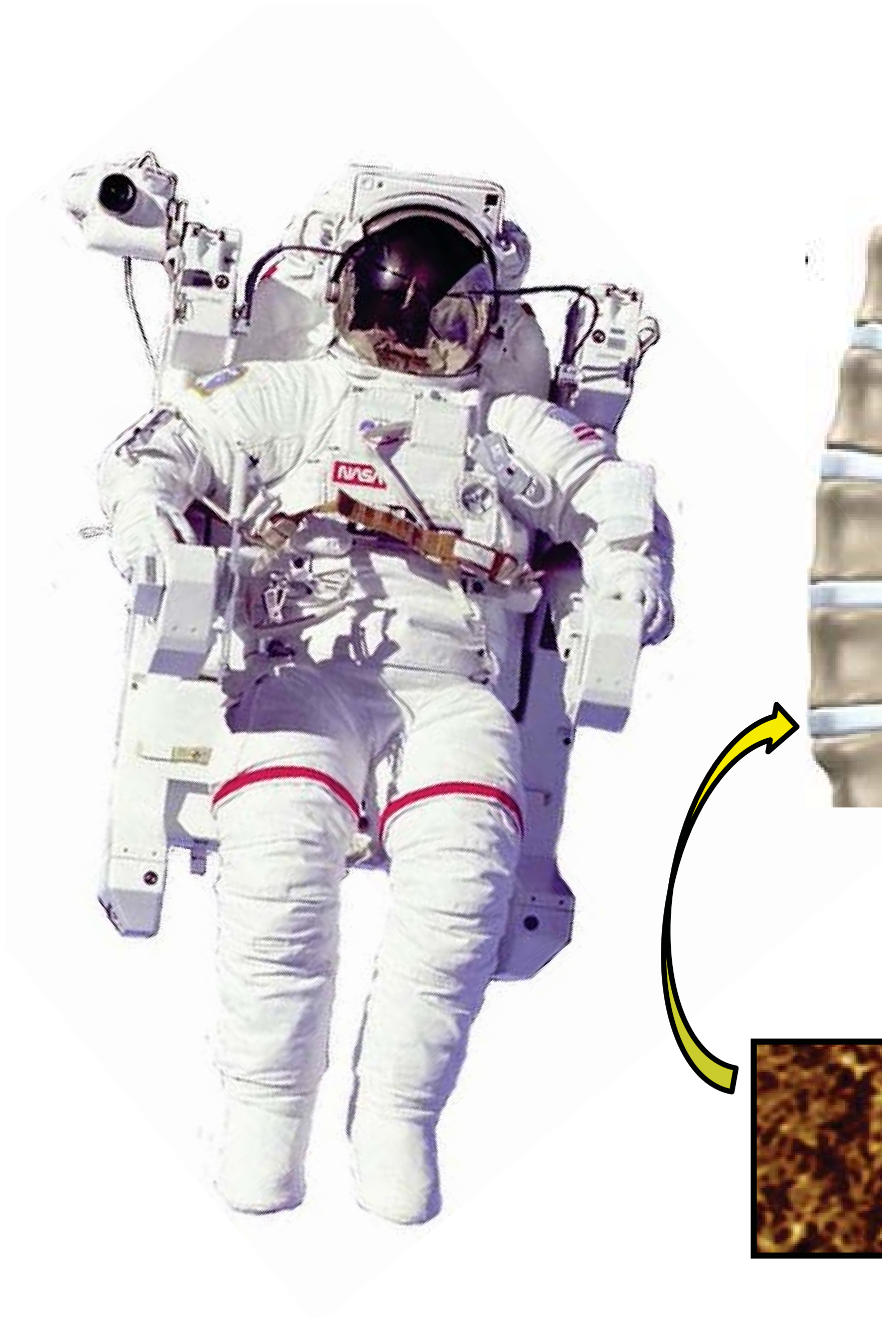
(secreted)

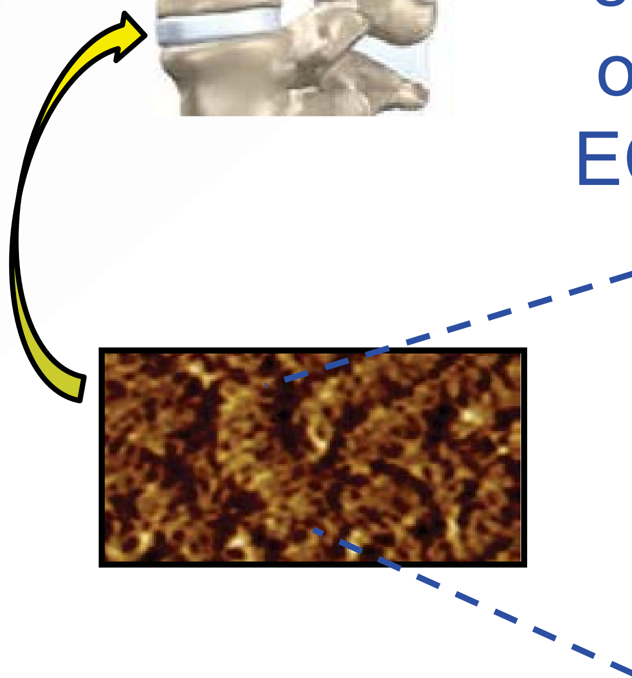
Brevican

Dr. Susan Hockfield

AGGRECAN: Resists Compression





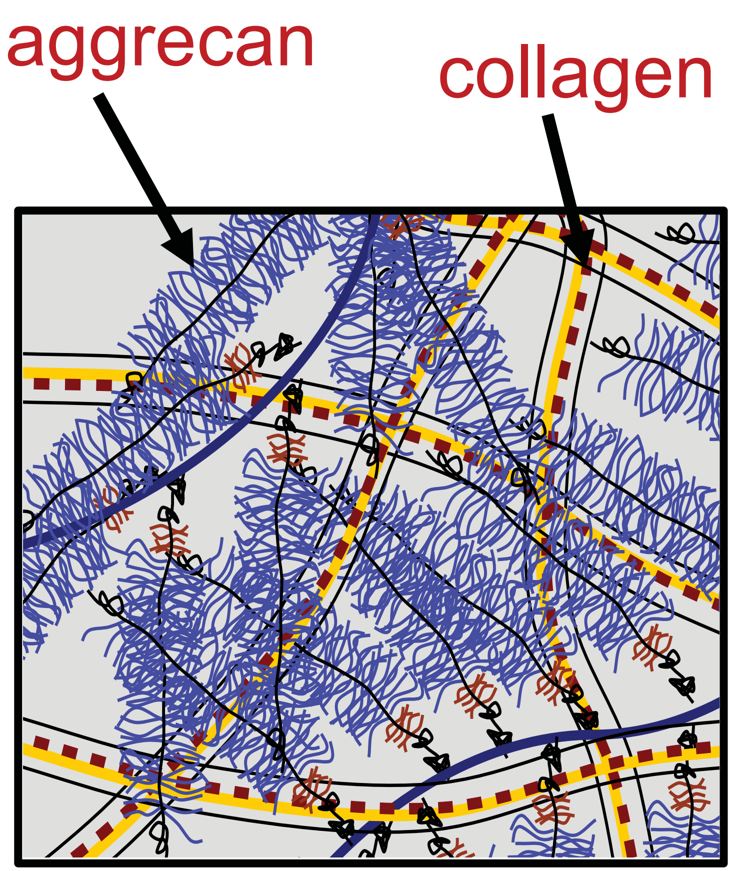


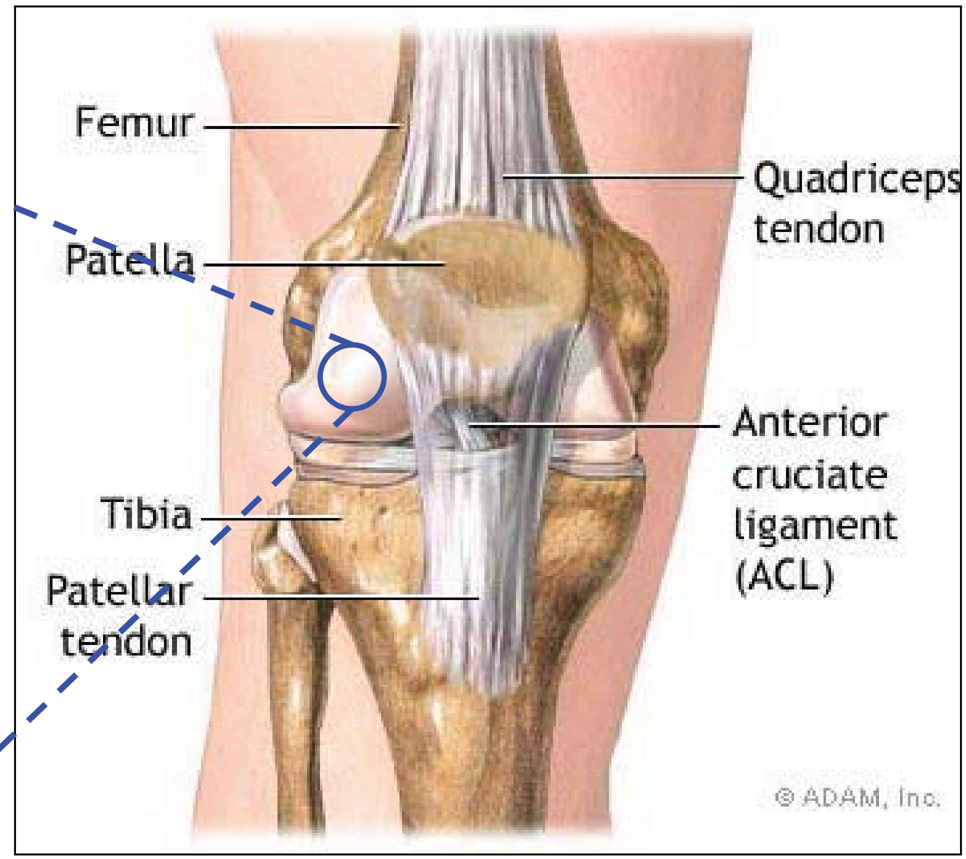
Astronauts gain
1-2 inches in height
during space flight:
swelling of the
intervertebral discs
under 0-gravity:

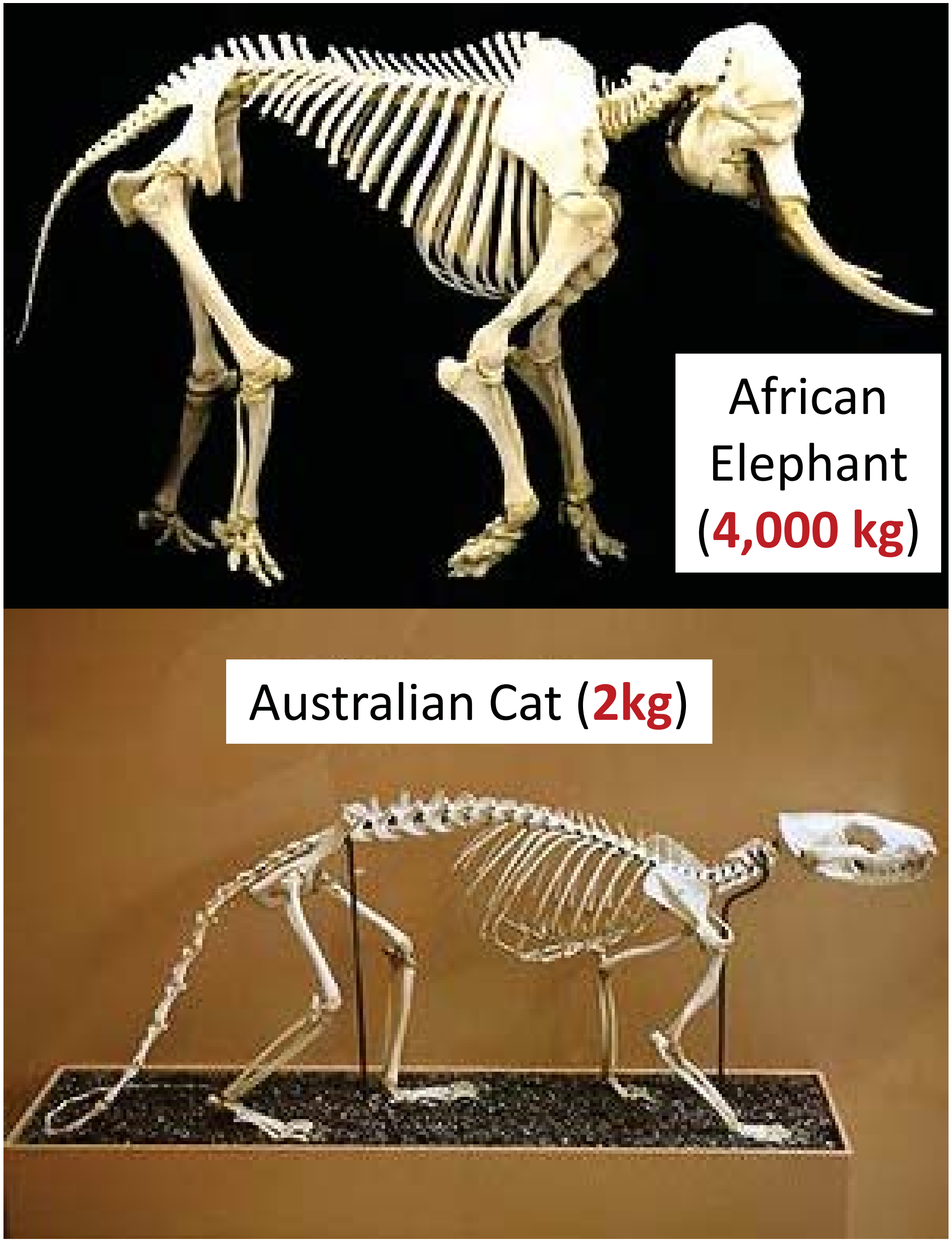
"swelling pressure"
of highly charged
ECM…aggrecan !!

Aggrecan: Resists Compression (in Cartilage)

Collagen: Resists Tension (in Cartilage)







Scaling of body size

Perfect Isometric scaling of organisms:

- Volume-based properties change proportionally to the body mass
- Surface area-based properties change with mass to the power 2/3
- Length-based properties change with mass to the 1/3 power

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Of Mice, Men and Elephants: The Relation between Articular Cartilage Thickness and Body Mass

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Abstract

Mammalian articular cartilage serves diverse functions, including shock absorption, force transmission and enabling low-friction joint motion. These challenging requirements are met by the tissue's thickness combined with its highly specific extracellular matrix, consisting of a glycosaminoglycan-interspersed collagen fiber network that provides a unique combination of resilience and high compressive and shear resistance. It is unknown how this critical tissue deals with the challenges posed by increases in body mass. For this study, osteochondral cores were harvested post-mortem from the central sites of both medial and lateral femoral condyles of 58 different mammalian species ranging from 25 g (mouse) to 4000 kg (African elephant). Joint size and cartilage thickness were measured and biochemical composition (glycosaminoclycan, collagen and DNA content) and collagen cross-links densities were analyzed. Here, we show that cartilage thickness at the femoral condyle in the mammalian species investigated varies between 90 µm and 3000 µm and bears a negative allometric relationship to body mass, unlike the isometric scaling of the skeleton. Cellular density (as determined by DNA content) decreases with increasing body mass, but gross biochemical composition is remarkably constant. This however need not affect life-long performance of the tissue in heavier mammals, due to relatively constant static compressive stresses, the zonal organization of the tissue and additional compensation by joint congruence, posture and activity pattern of larger mammals. These findings provide insight in the scaling of articular cartilage thickness with body weight, as well as in cartilage biochemical composition and cellularity across mammalian species. They underscore the need for the use of appropriate *in vivo* models in translational research aiming at human applications.

Species

1	Mouse (*Mus Musculus*)
2	Pygmy marmoset (*Callithrix pygmaea*)
3	Common marmoset (*Callithrix jacchus*)
4	Rat (*Rattus sp.*)
5	Cotton-top or Pinché tamarin (*Saguinus oedipus*)
6	Eurasian Red squirrel (*Sciurus vulgaris*)
7	Cape Ground squirrel (*Xerus inauris*)
8	Guinea pig (*Cavia porcellus*)
9	Potto (*Perodicticus potto*)
10	Ferret (*Mustela putorius furo*)
11	White-faced saki (*Pithecia pithecia*)
12	Ring-tailed lemur (*Lemur catta*)
13	Opossum (*Didelphis sp.*)
14	Oriental small-clawed otter (*Aonyx cinerea*)
15	Hare (*Lepus sp.*)
16	Rabbit (*Oryctolagus cuniculus*)
17	South American coati (*Nasua Nasua*)
18	European otter (*Lutra lutra*)
19	Linnaeus's two-toed sloth (*Choloepus didactylus*)
20	Black Mangabey (*Lophocebus albigena*)
21	Vervet monkey (*Chlorocebus pygerythrus*)
22	Southern or Chilean Pudú (*Pudu puda*)
23	Woolly Monkey (*Lagothrix lagotricha*)
24	Barbary macaque (*Macaca sylvanus*)
25	Badger (*Meles meles*)
26	Dikdik (*Madoqua kirkii*)
27	Beagle dog (*Canis sp.*)
28	Tammar wallaby (*Macropus eugenii*)
29	Hamadryas baboon (*Papio hamadryas*)
30	Indian crested porcupine (*Hystrix indica*)
31	Thompson's gazelle (*Eudorcas thomsoni*)
32	Roe deer (*Capreolus capreolus*)
33	Capybara (*Hydrochoerus hydrochaeris*)
34	Gorilla (*Troglodytes gorilla*)
35	Dutch milk goat (*Capri hircus*)
36	West African dwarf goat (*Capri sp.*)
37	Cheetah (*Acinonyx jubatus*)
38	Impala (*Aepyceros melampus*)
39	Red Kangaroo (*Macropus rufus*)
40	Human (*Homo Sapiens*)
41	Fallow deer (*Dama dama*)
42	Siberian tiger (*Pathera tigris*)
43	Reindeer (*Rangifer tarandus*)
44	Lion (*Panthera leo*))
45	Horse (mini-shetland) (*Equus sp.*)
46	Kudu (*Tragelaphus strepsiceros*)
47	Llama (*Lama Glama*)
48	Polar bear (*Ursus Maritimus*)
49	South American tapir (*Tapirus terrestris*)
50	European moose (*Alces alces alces*)
51	Watoessi (*Bos Taurus Taurus watussi*)
52	Dairy cow (*Bovinae*)
53	Giraffe (*Giraffa camleopardalis*)
54	Horse (*Equus ferus caballus*)
55	Banteng (*Bos javanicus*)
56	White rhinoceros (*Ceratotherium simum*)
57	Asian elephant (*Elaphus maximus*)

Of Mice, Men and Elephants:

.....Cartilage Thickness and Body Mass.....

University Medical Center,
Utrecht,
The Netherlands

How does cartilage size & biochemical content scale with increasing animal size?

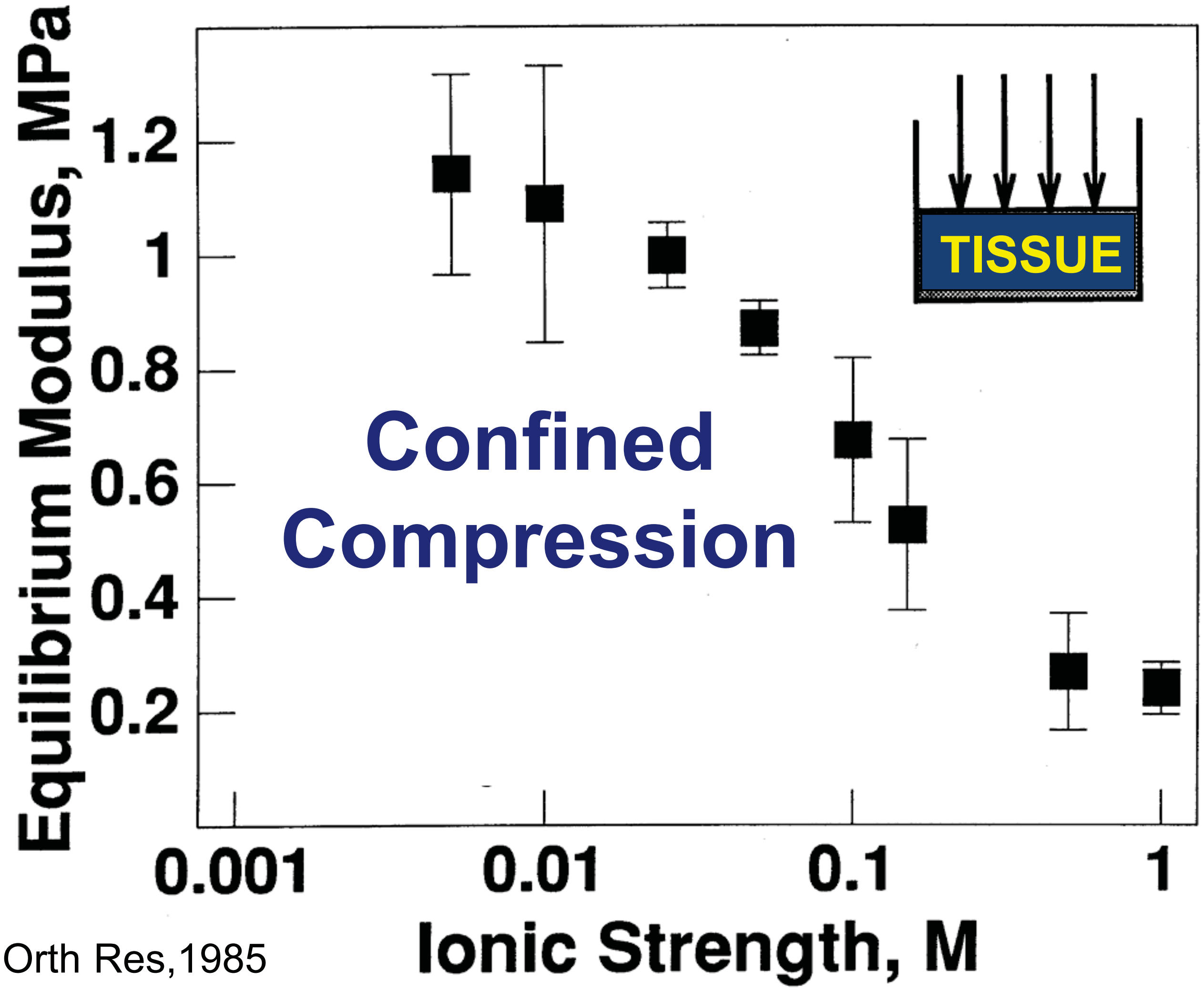
Google: “elastic moduli”

Every elastic modulus can be expressed in terms of two other moduli

	bulk	Young’s	Lamé #2	Shear	Poisson	Longitudinal
	$K =$	$E =$	$\lambda =$	$G =$	$\nu =$	$M =$ H
$(K,\, E)$	K	E	$\frac{3K(3K-E)}{9K-E}$	$\frac{3KE}{9K-E}$	$\frac{3K-E}{6K}$	$\frac{3K(3K+E)}{9K-E}$
$(K,\, \lambda)$	K	$\frac{9K(K-\lambda)}{3K-\lambda}$	λ	$\frac{3(K-\lambda)}{2}$	$\frac{\lambda}{3K-\lambda}$	$3K - 2\lambda$
$(K,\, G)$	K	$\frac{9KG}{3K+G}$	$K - \frac{2G}{3}$	G	$\frac{3K-2G}{2(3K+G)}$	$K + \frac{4G}{3}$
$(K,\, \nu)$	K	$3K(1-2\nu)$	$\frac{3K\nu}{1+\nu}$	$\frac{3K(1-2\nu)}{2(1+\nu)}$	ν	$\frac{3K(1-\nu)}{1+\nu}$
$(K,\, M)$	K	$\frac{9K(M-K)}{3K+M}$	$\frac{3K-M}{2}$	$\frac{3(M-K)}{4}$	$\frac{3K-M}{3K+M}$	M
$(E,\, \lambda)$	$\frac{E+3\lambda+R}{6}$	E	λ	$\frac{E-3\lambda+R}{4}$	$\frac{2\lambda}{E+\lambda+R}$	$\frac{E-\lambda+R}{2}$
$(E,\, G)$	$\frac{EG}{3(3G-E)}$	E	$\frac{G(E-2G)}{3G-E}$	G	$\frac{E}{2G} - 1$	$\frac{G(4G-E)}{3G-E}$
$(E,\, \nu)$	$\frac{E}{3(1-2\nu)}$	E	$\frac{E\nu}{(1+\nu)(1-2\nu)}$	$\frac{E}{2(1+\nu)}$	ν	$\frac{E(1-\nu)}{(1+\nu)(1-2\nu)}$
$(\lambda,\, G)$	$\lambda + \frac{2G}{3}$	$\frac{G(3\lambda+2G)}{\lambda+G}$	λ	G	$\frac{\lambda}{2(\lambda+G)}$	$\lambda + 2G$

Static Equilibrium Modulus

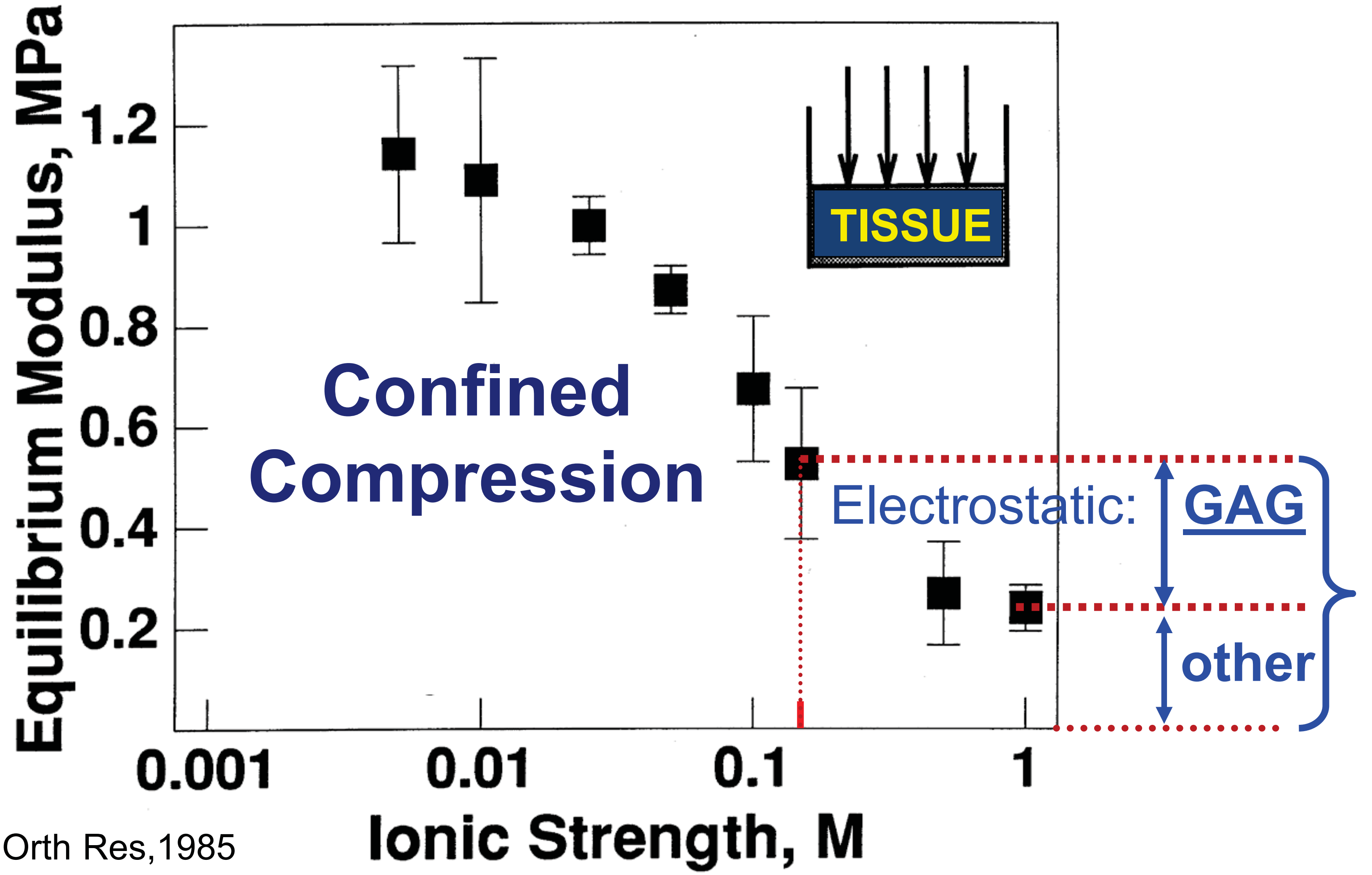
(Adult bovine cartilage)



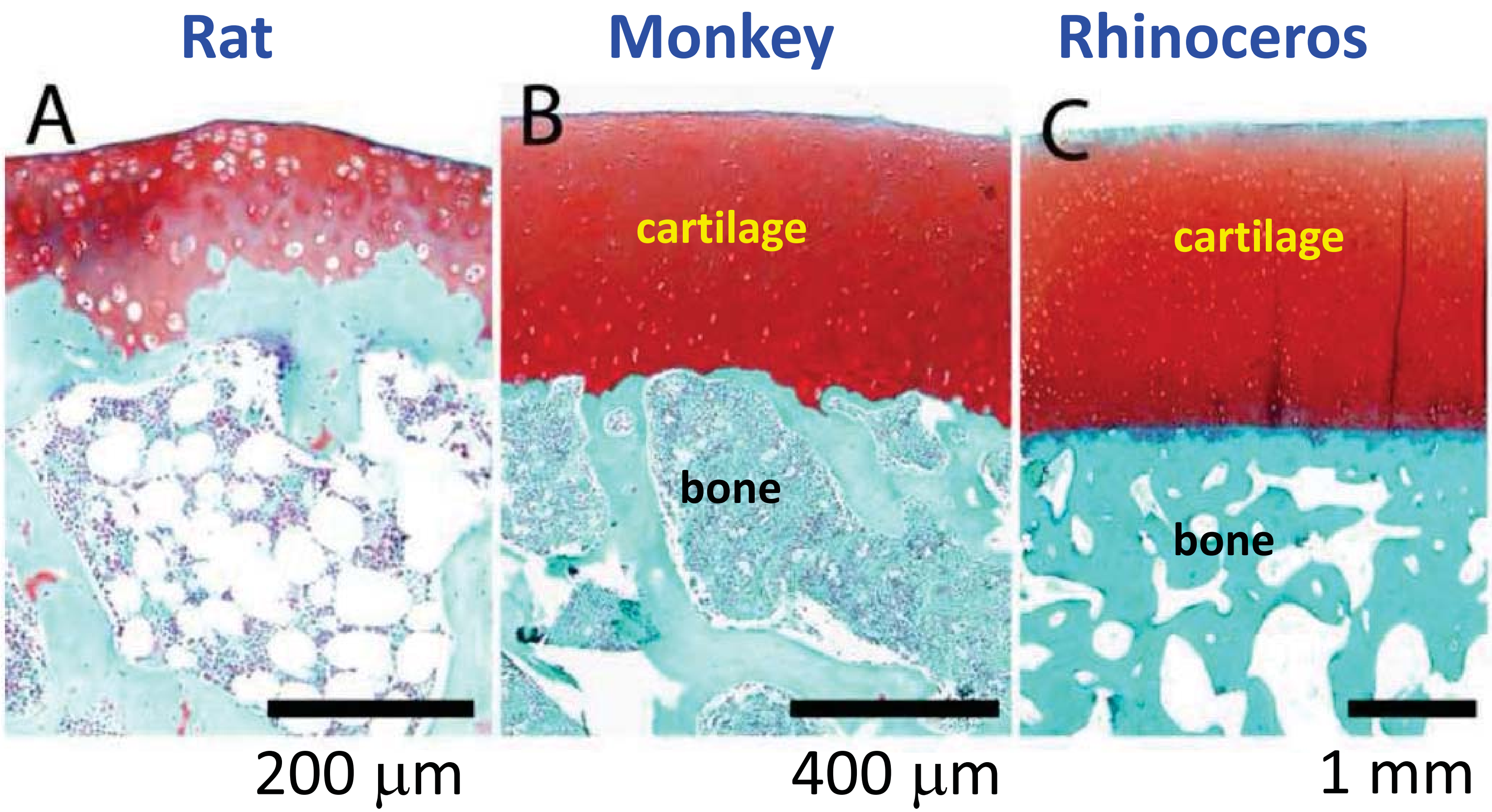
Eisenberg, J Orth Res,1985

Static Equilibrium Modulus

(Adult bovine cartilage)

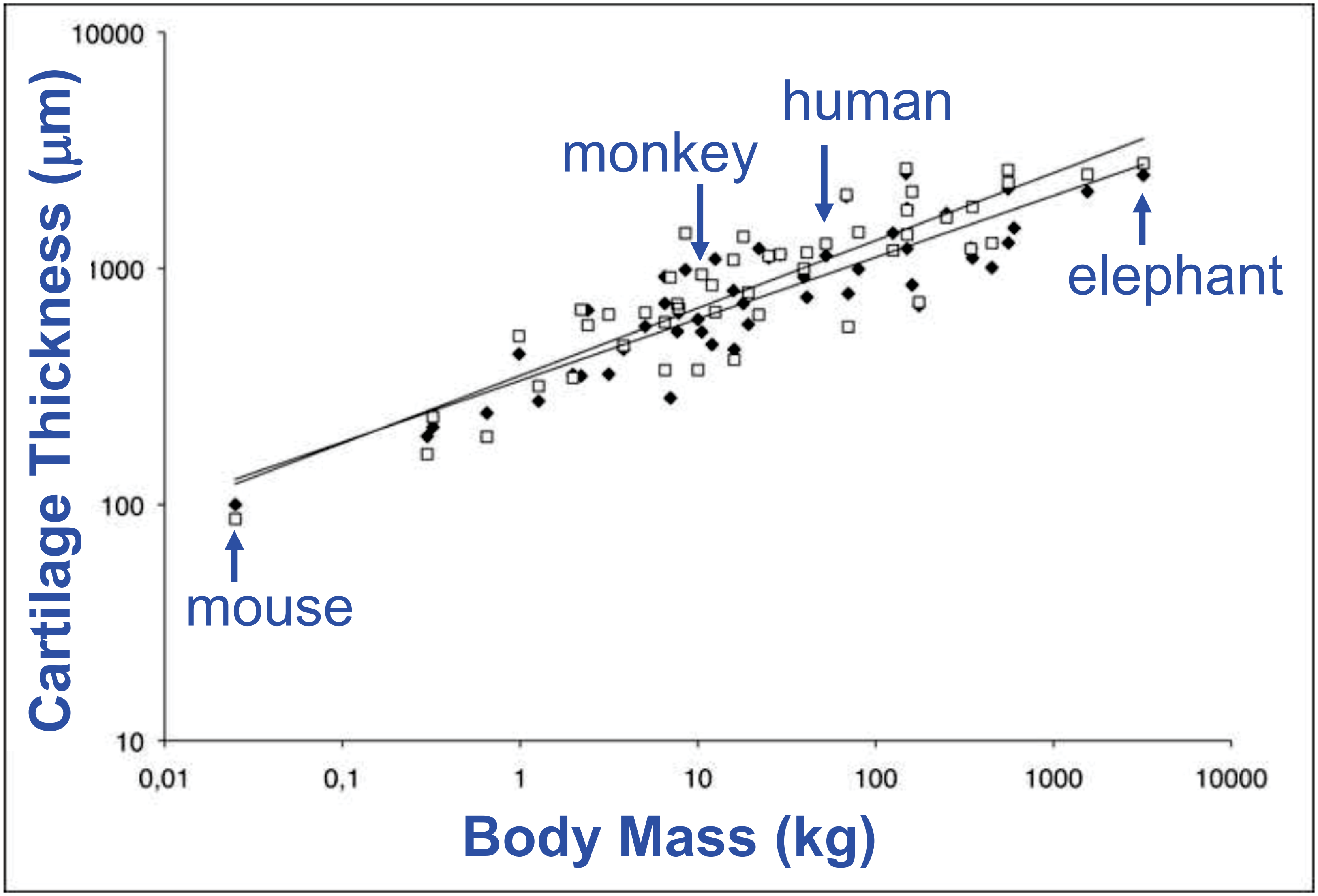


Eisenberg, J Orth Res,1985



"Safranin-O" (red) stains Glycosaminoglycans
(of Proteoglycans)

Source: Malda, Jos, et al. "Of Mice, Men and Elephants: The Relation between Articular Cartilage Thickness and Body Mass." *PloS one* 8, no. 2 (2013): e57683.



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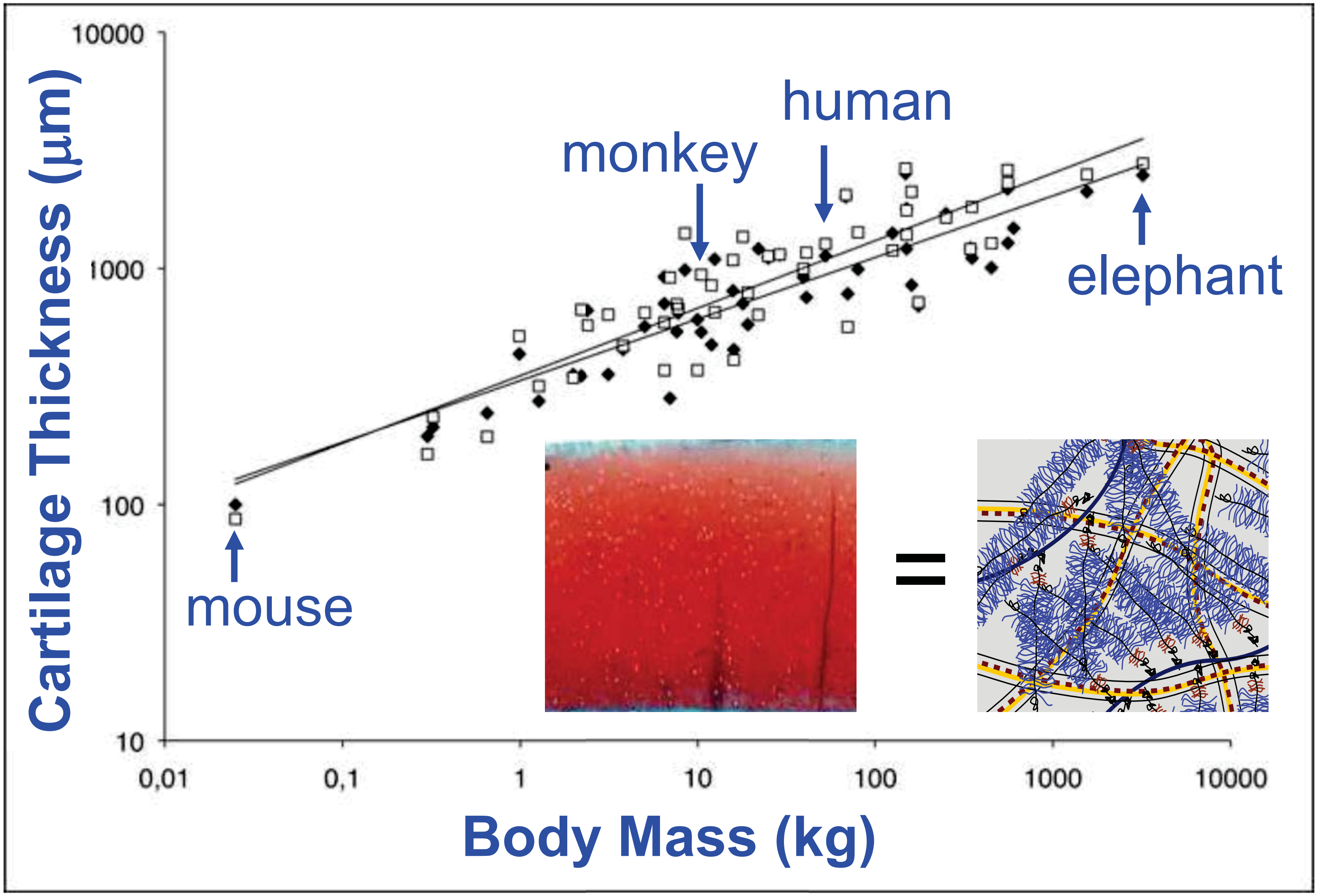

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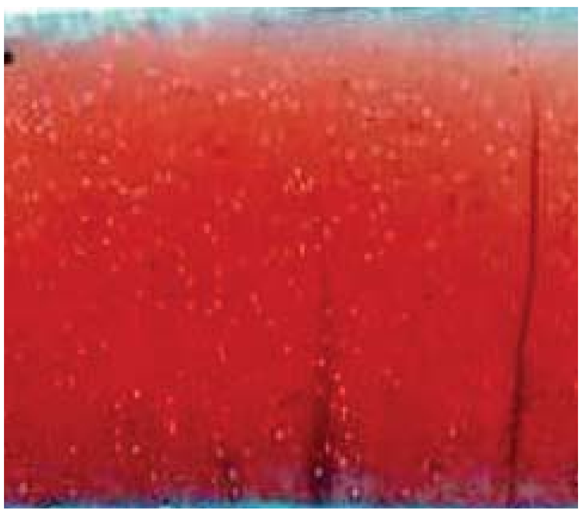
Source: Malda, Jos, et al. "Of Mice, Men and Elephants: The Relation between Articular Cartilage Thickness and Body Mass." *PloS one* 8, no. 2 (2013): e57683.

Tensile & Shear Modulus: Collagen

Compressive Modulus: Aggrecan-GAGs

→ (**Modulus**: an "**intrinsic material property**" .....**independent of size, shape**....)



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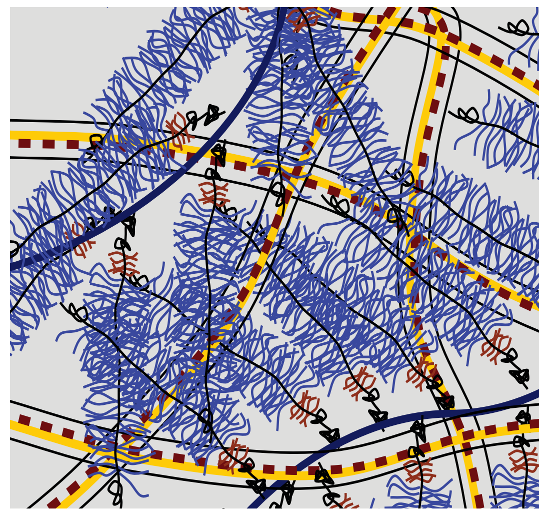


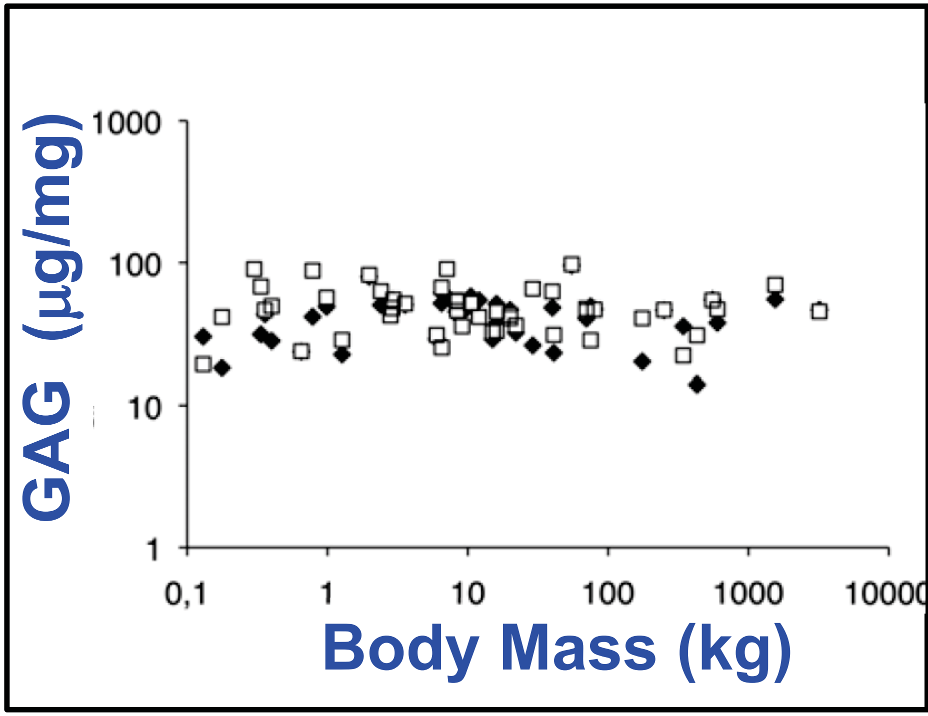
Aggrecan -GAGs

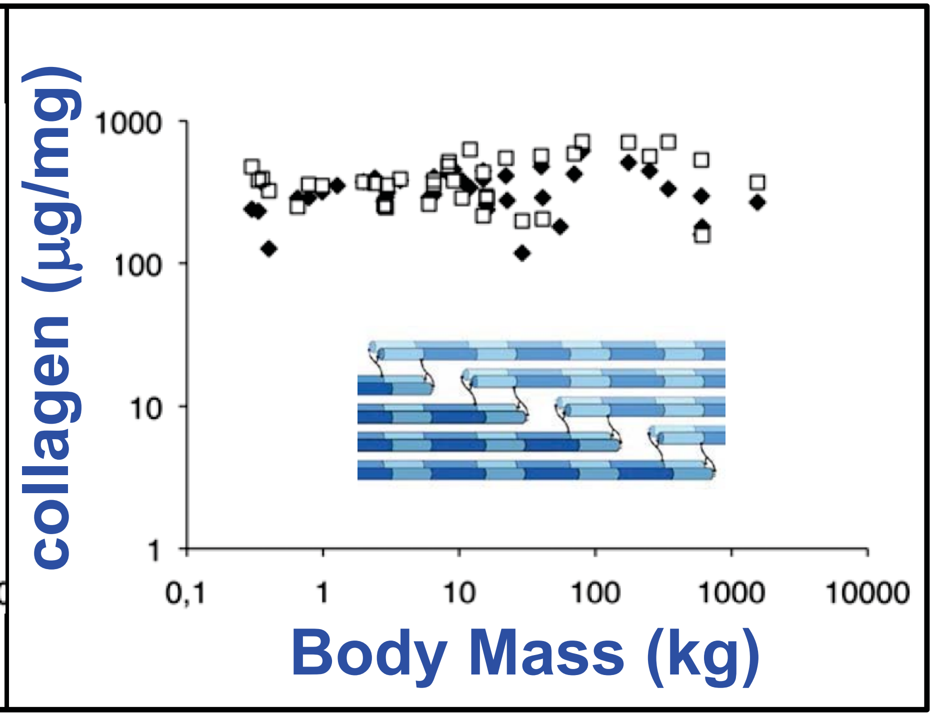
Collagen

Tensile & Shear Modulus: Collagen

Compressive Modulus: Aggrecan-GAGs

(Modulus: an "intrinsic material property" .....independent of size, shape....)





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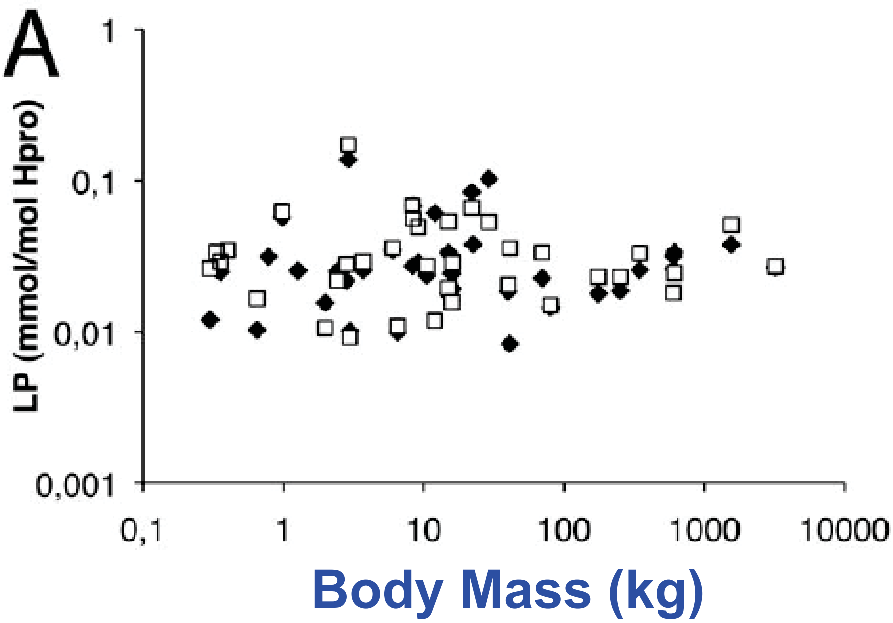
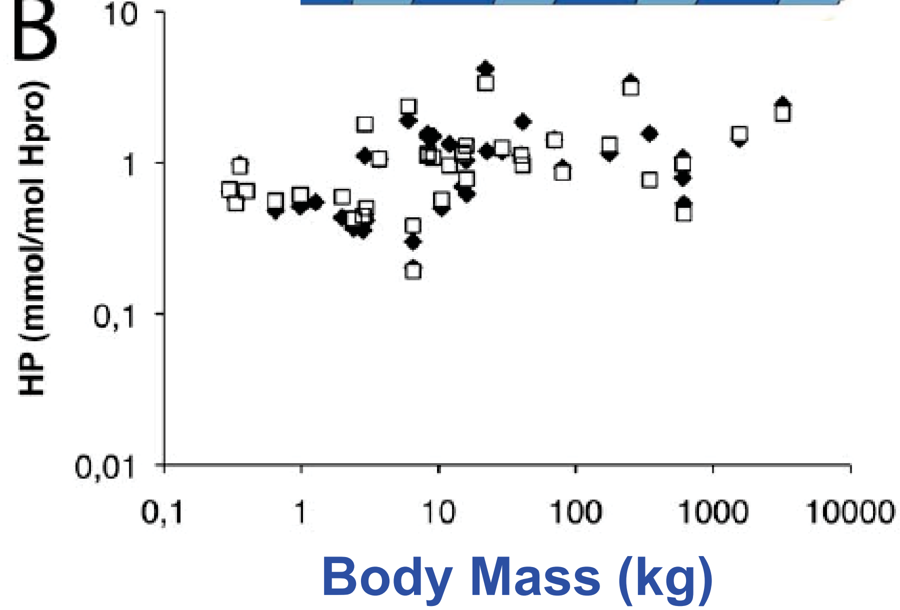
Source: Malda, Jos, et al. "Of Mice, Men and Elephants: The Relation between Articular Cartilage Thickness and Body Mass." *PloS one* 8, no. 2 (2013): e57683.

Figure 5. Average collagen cross-link content

(A) Lysyl-pyridinoline and (B) hydroxylsyl-pyridinoline cross-links are **independent of body mass**

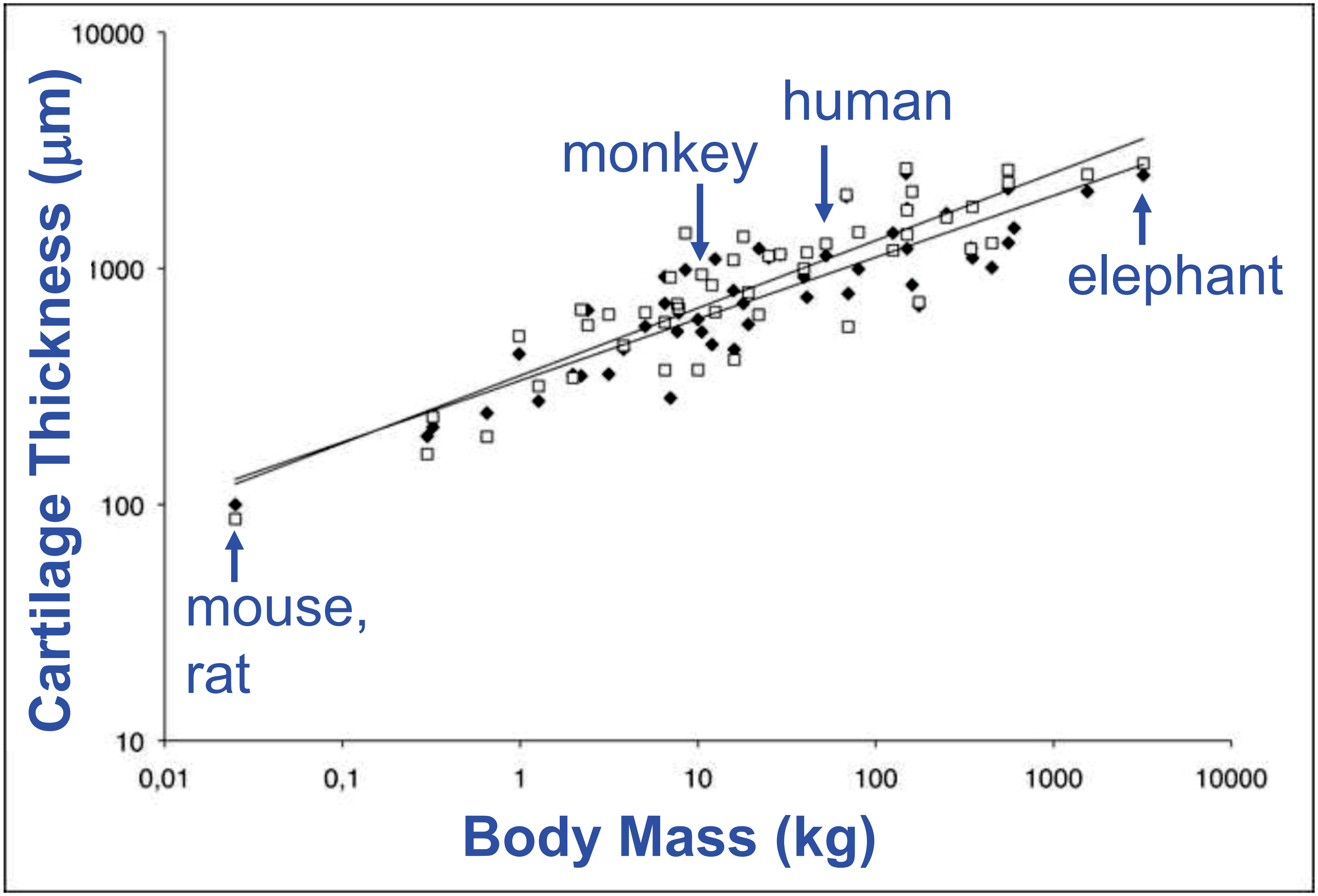




Lysyl-pyridinoline x-links

hydroxylsyl-pyridinoline x-links

Source: Malda, Jos, et al. "Of Mice, Men and Elephants: The Relation between Articular Cartilage Thickness and Body Mass." *PloS one* 8, no. 2 (2013): e57683.



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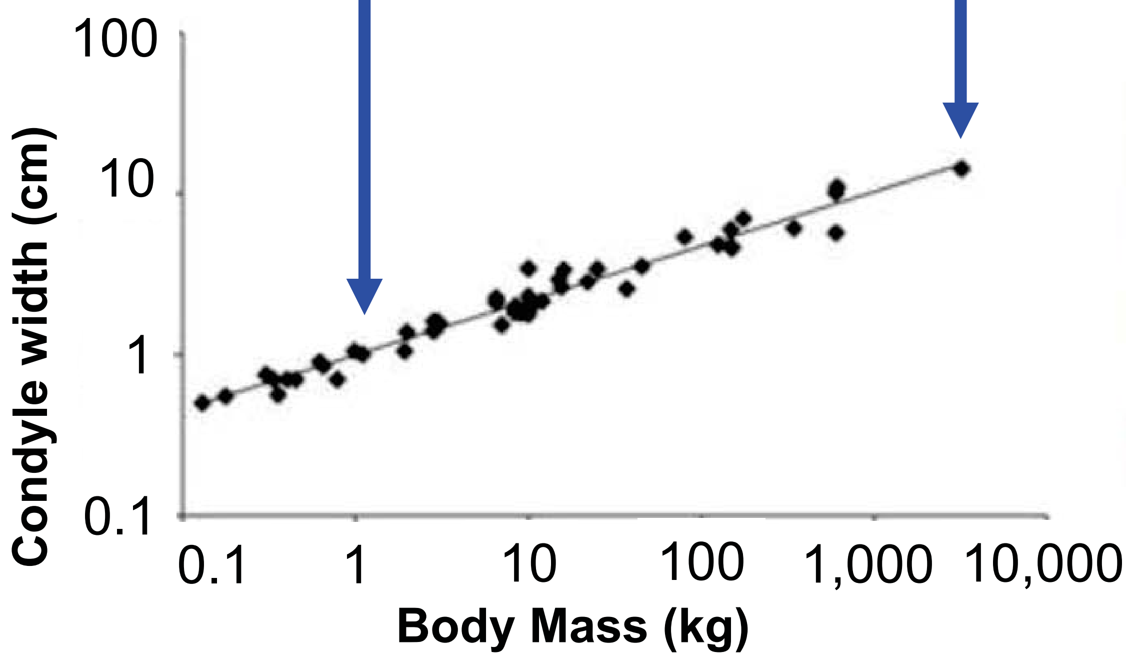
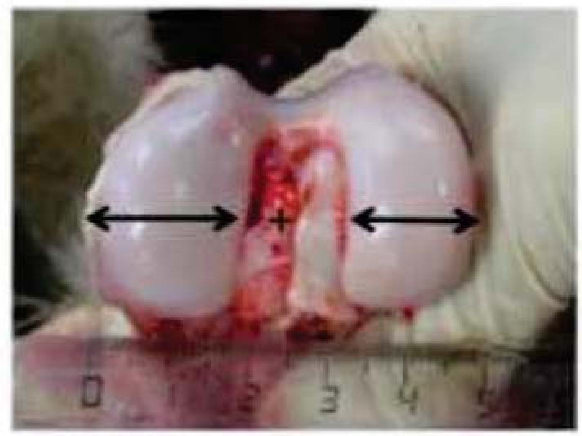
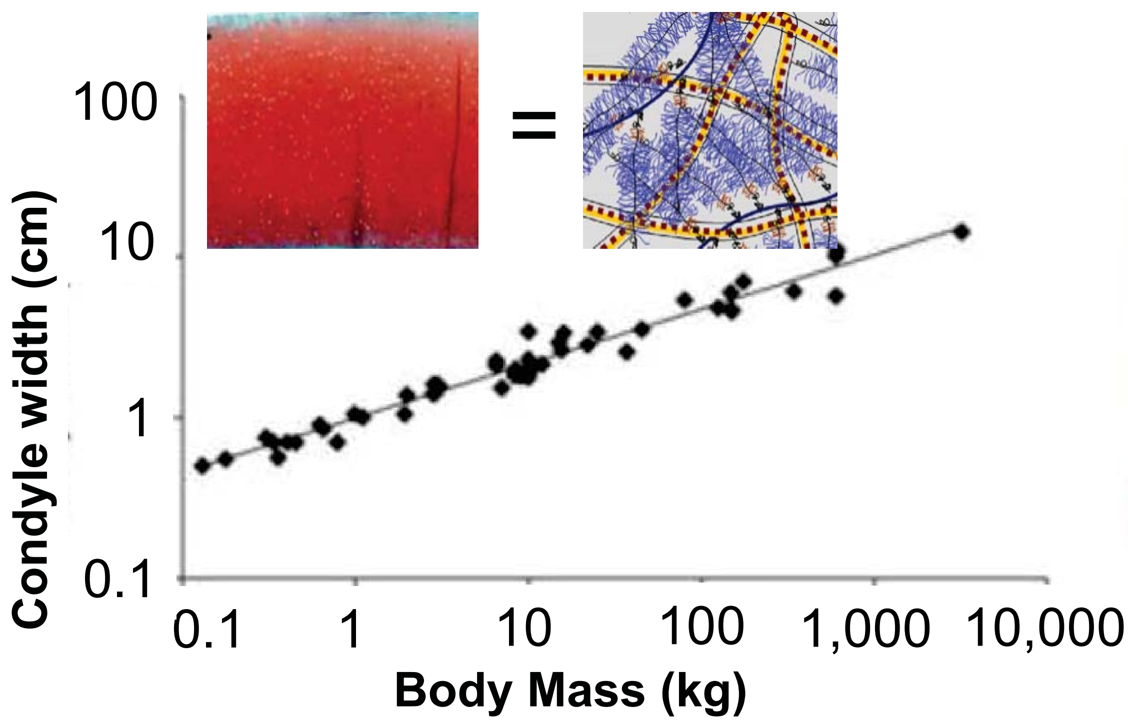

100
10
1
0.1
Condyle width (cm)
0.1
1
10
100
1,000
10,000
Body Mass (kg)

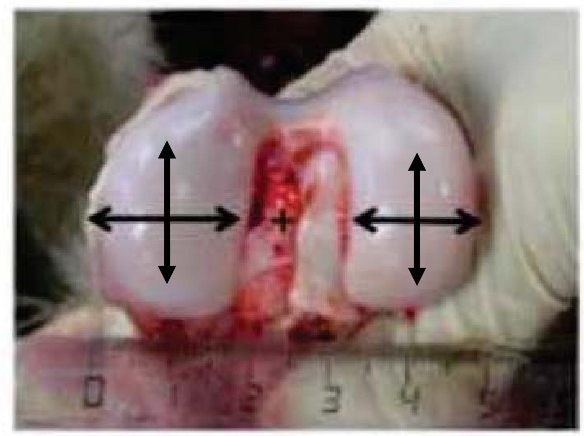


Loading Area $\propto$ $[\text{width}]^2$ **scales as** $(\textbf{Body Mass})^{\frac{2}{3}}$

Stress = **(Force/Area)** on Joint surface is **~ Same**

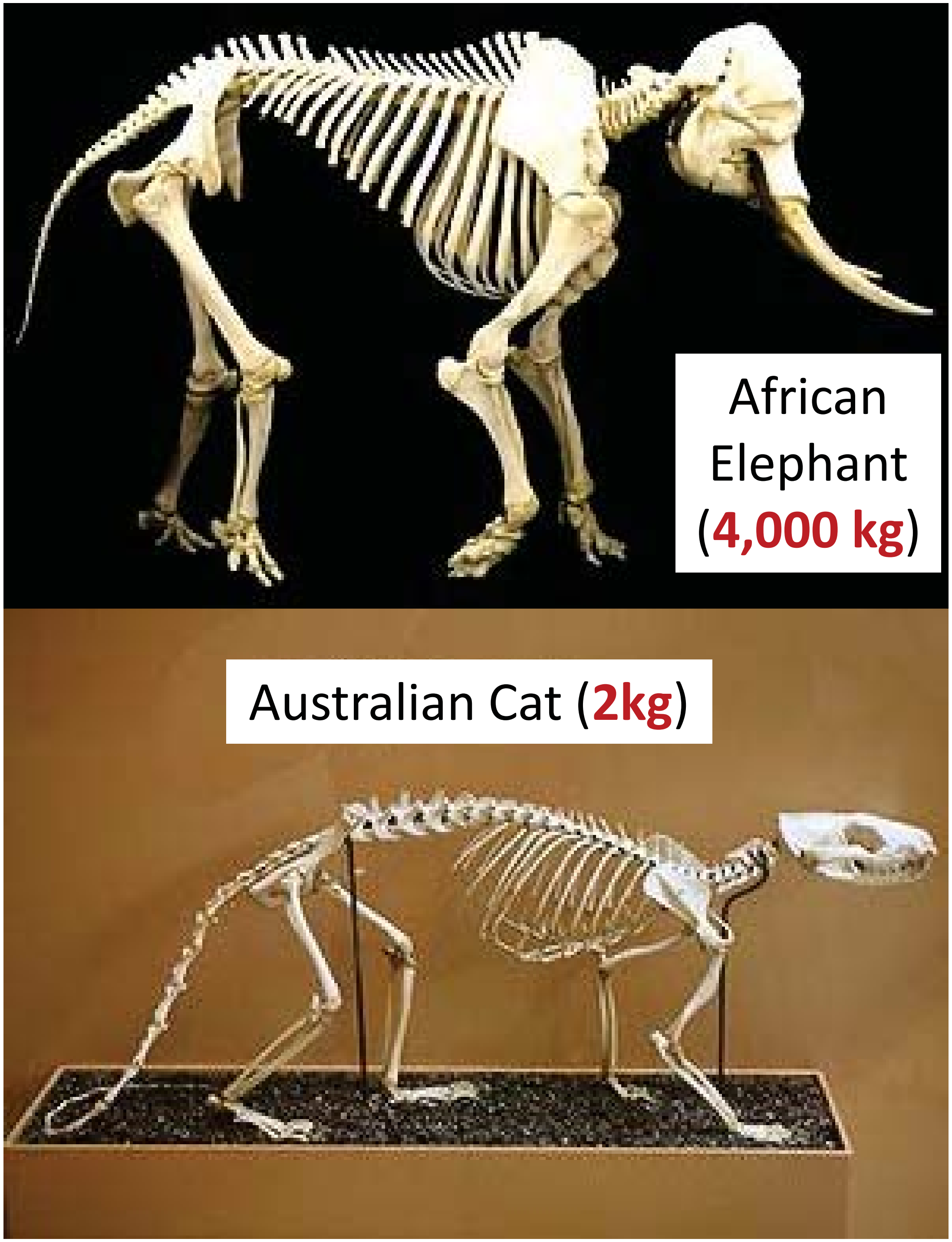
$\therefore$ **cells have optimized the appropriate ECM**





"$\sigma \simeq E_{cart}\, \varepsilon$"

Source: Malda, Jos, et al. "Of Mice, Men and Elephants: The Relation between Articular Cartilage Thickness and Body Mass." *PloS one* 8, no. 2 (2013): e57683.



Scaling of body size

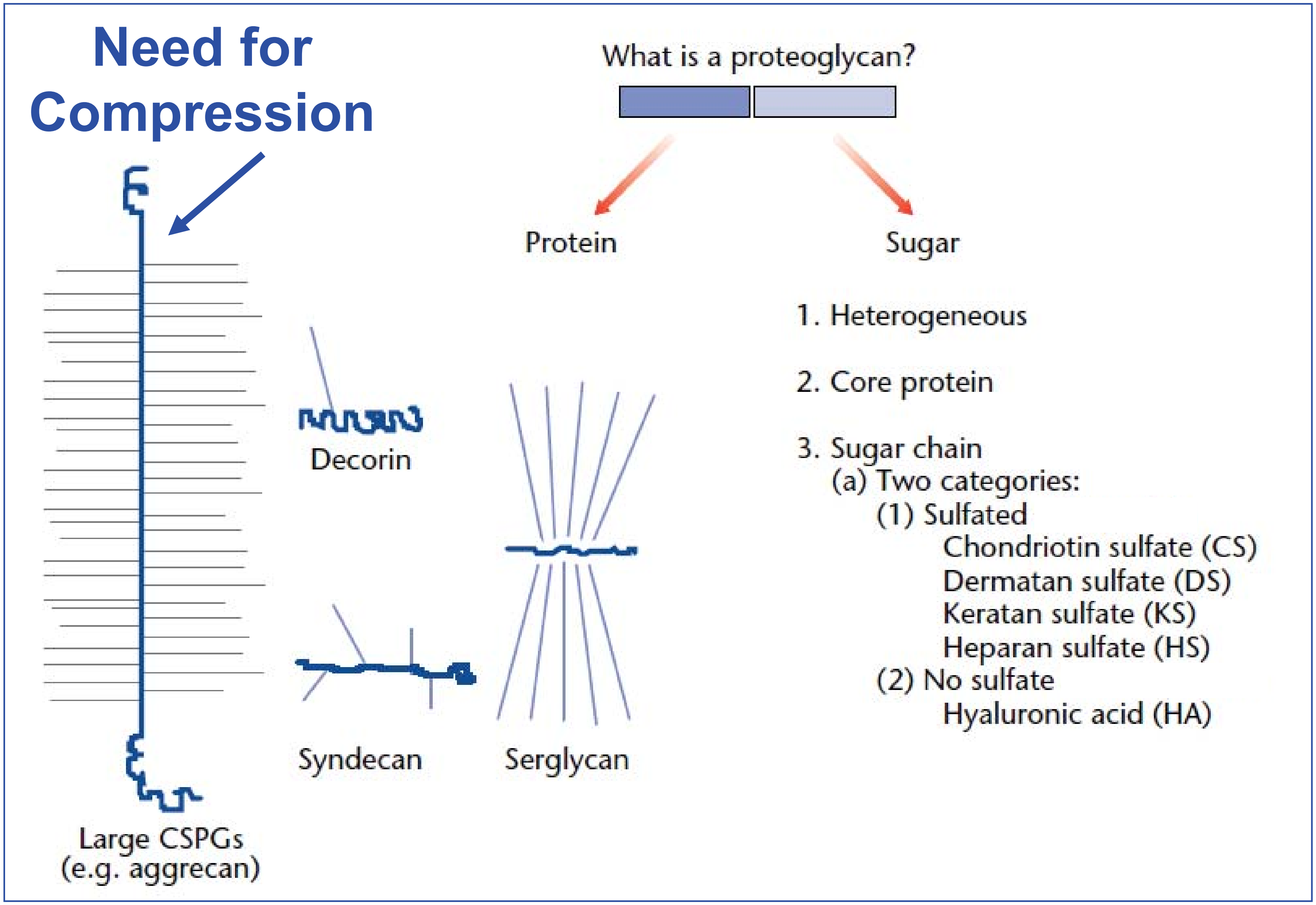
Perfect Isometric scaling of organisms:

- Volume-based properties change proportionally to the body mass
- <u>Surface area-based properties change with mass to the power 2/3</u>
- Length-based properties change with mass to the 1/3 power

Proteoglycans

Encyclop Life Sci, 2009

Nancy B Schwartz, *University of Chicago, Illinois, USA*



Source: Schwartz, Nancy B. "Proteoglycans." eLS (2009).

EM: (1980's)

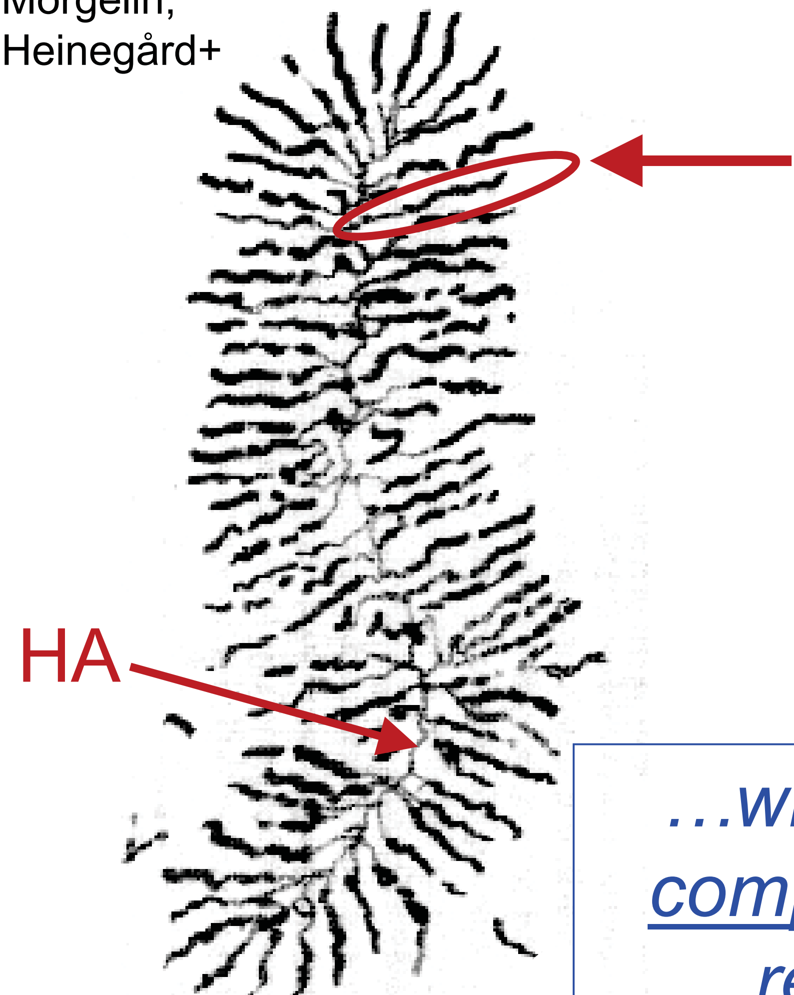
Buckwalter, Rosenberg+

Mörgelin, Heinegård+

Aggrecan Monomer

(Bovine Fetal Epiphyseal)

(Ng+, J. Struc Biol, 2003)



“Aggregate”

GAG Chains

Core Protein

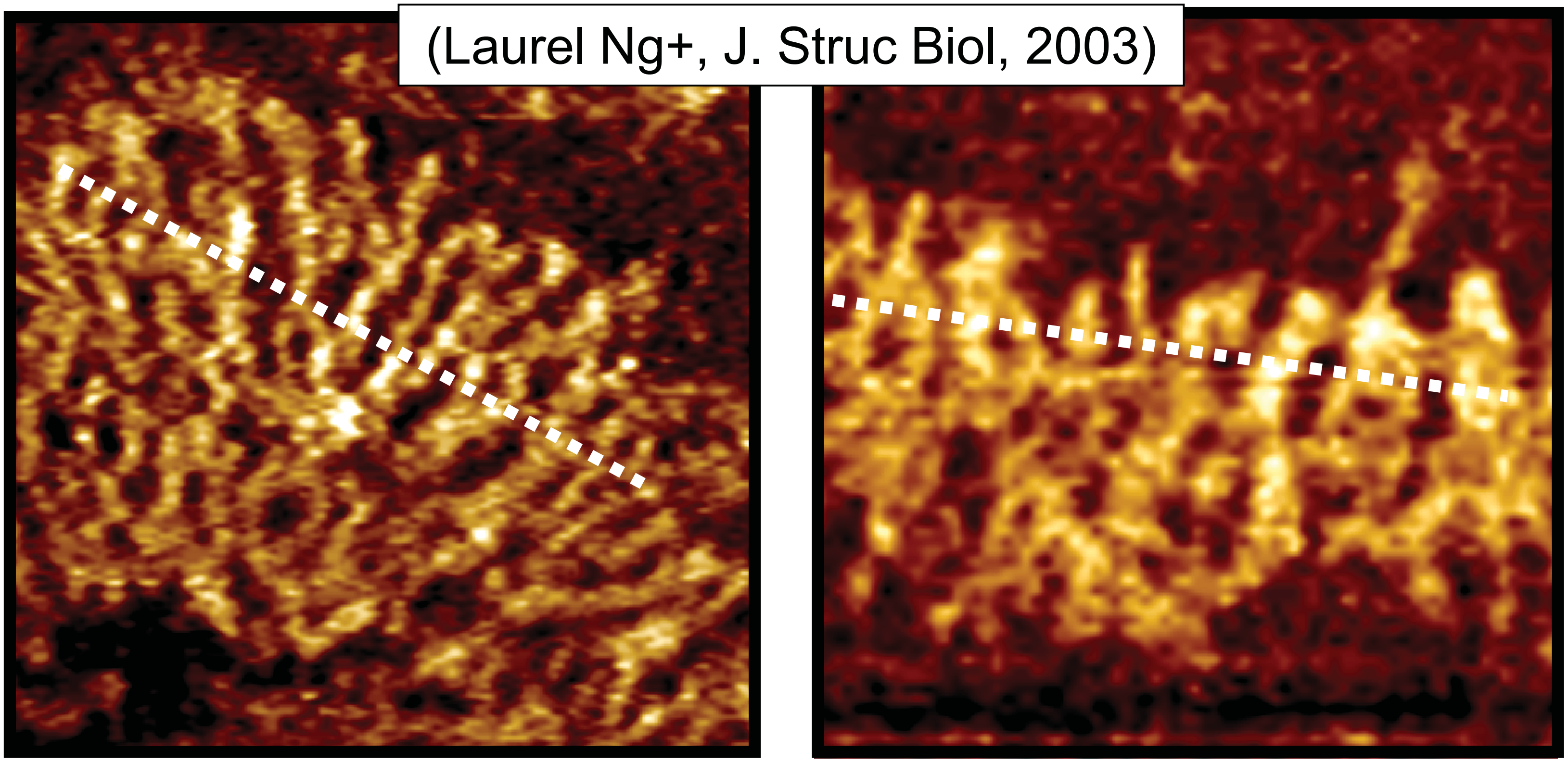
50 nm

(Tapping mode AFM)

…why does the cell make such a ***complicated****,* ***charged*** *molecule to resist compressive loads??*

(cells in cartilage, disc, tendon, many other tissues)

Source of Electrostatic Interactions (330 ☺)



Fetal Epiphyseal (Bovine) Adult - Nasal

Courtesy Elsevier, Inc., http://www.sciencedirect.com. Used with permission.
Source: Ng, Laurel, et al. "Individual Cartilage Aggrecan Macromolecules and their Constituent Glycosaminoglycans Visualized via Atomic Force Microscopy." *Journal of Structural Biology* 143 (2003): 242-57.

$L_{contour}$ = 41 ± 7 nm 32 ± 5 nm

GAG Spacing = 3.2 ± 0.8 nm 4.4 ± 1.2 nm

(Debye Length ~ 1 nm at physiological ionic strength)

Stan Sajdera **Vince Hascall**

c. 1969

The Journal of Biological Chemistry
Vol. 244, No. 1, Issue of January 10, pp. 77–87, 1969
Printed in U.S.A.

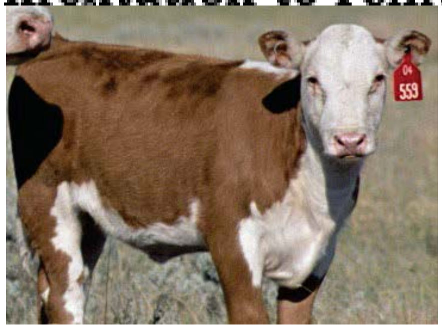
1969 J Biol Chem

Proteinpolysaccharide Complex from Bovine Nasal Cartilage

A COMPARISON OF LOW AND HIGH SHEAR EXTRACTION PROCEDURES*

Stanley W. Sajdera‡ and Vincent C. Hascall‡
From The Rockefeller University, New York, New York 10021

Two procedures for isolating 80 to 85% of the total hexuronic acid from bovine nasal cartilage as proteinpolysaccharide complex are described and compared.
cetylpyridinium salt. The second method, termed dissociative, avoids shear by extracting proteinpolysaccharides into solvents containing optimal concentrations of various electrolytes and then utilizes equilibrium density gradient sedimentation to remove glycoprotein and soluble collagen.



Guanidinium chloride-extracted PPC (PPC(Gu)) was prepared by extracting cartilage slices with 15 volumes of 4.0 M guanidinium chloride, 0.05 M Tris-HCl, pH 7.5, at room temperature with magnetic stirring for 24 hours.

Laurel Ng, J. Struc Biol, 2003: **deleted from Methods before publication ☺**

Preparation of Aggrecan Monomers from Bovine Nasal Cartilage

MATERIALS:

GdmHCl, sodium acetate, protease inhibitors and CsCl were purchased from Aldrich Sigma Chemical Co, St. Louis MO. Dialysis membranes (Spectrapore, 54 mm flat width, a polycarbonate face shield, and the cryo apron and safety gloves were obtained through Fisher Scientific. A chain saw (12 hp), a fully equipped tool-box and extra strength garbage bags were purchased from Sears, Warwick Mall, Providence RI.

TISSUE PROCUREMENT:

Eight bovine heads were collected from the local slaughterhouse, placed on wet ice and, at midnight, transported in the trunk of a 1969 Chevy Caprice, to a 2-car-gargage situated on the **unoccupied property** of 64 Alfred Stone Road Providence, RI. Bovine heads were removed from the ice and placed, noses up, on the concrete floor before applying a through rinse with water using a garden hose. Heads were then individually immobilized in a vice, and……**nasal septum removed…………using a chain saw**. Nasal cartilages were removed, washed 3 times in ice-cold 50 mM sodium acetate…..

AGGRECAN EXTRACTION AND PURIFICATION:

Pooled nasal cartilages (~0.5 kg wet weight) were …. extracted at 4°C for 48 hours with in 5 L of **4 M guanidine HCl**, 100 mM sodium acetate, pH 7.0…….

20.310J / 3.053J / 6.024J / 2.797J Molecular, Cellular, and Tissue Biomechanics
Spring 2015