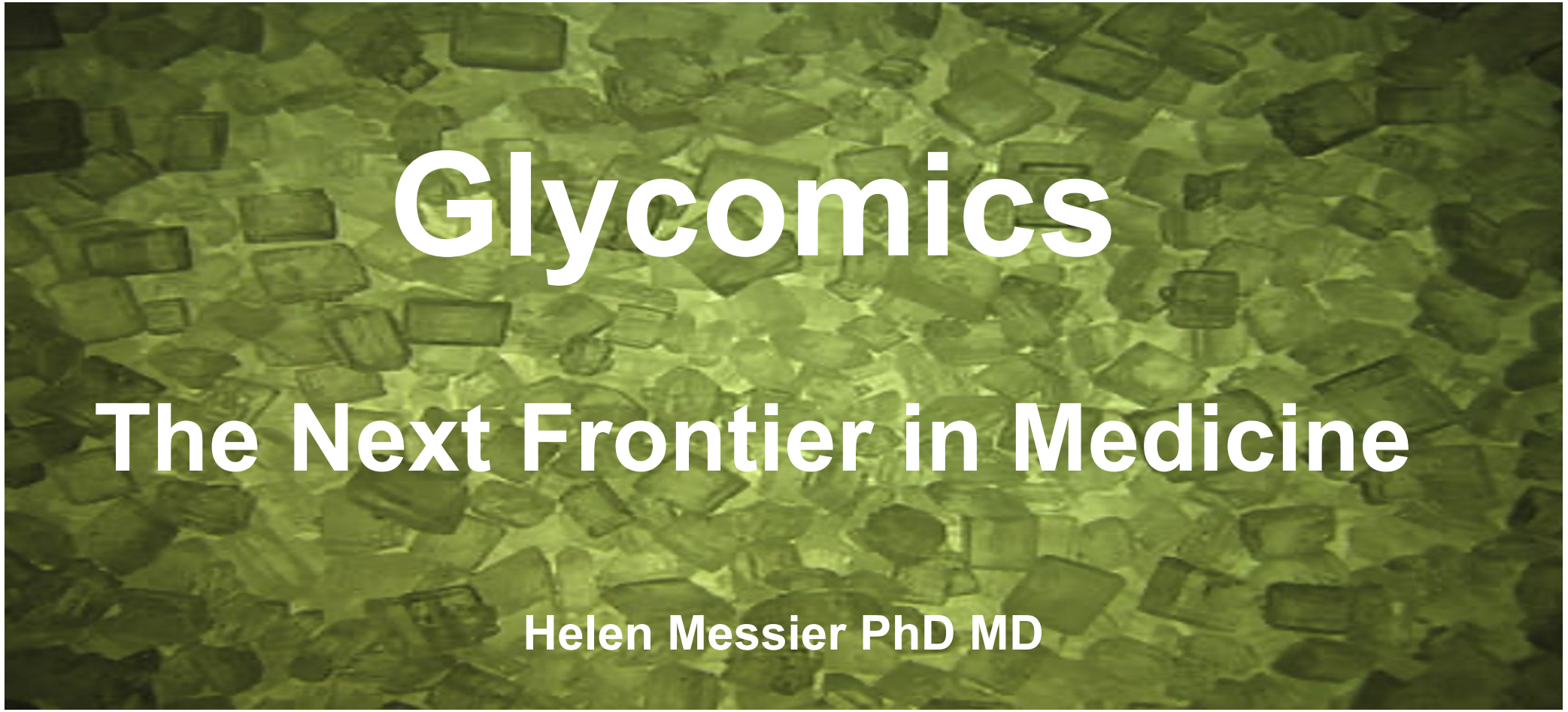
CALROY
HEALTH SCIENCES



Glycomics

The Next Frontier in Medicine

Helen Messier PhD MD

What do these have in common?

- HIV infection
- Cardiovascular disease
- H. pylori infection
- Rheumatoid arthritis
- SARS-CoV-2 susceptibility and infection
- Diabetes
- Kidney function and disease
- Modulation of the inflammatory response
- Cancer metastasis
- Regulation of apoptosis
- Seaweed

Objectives

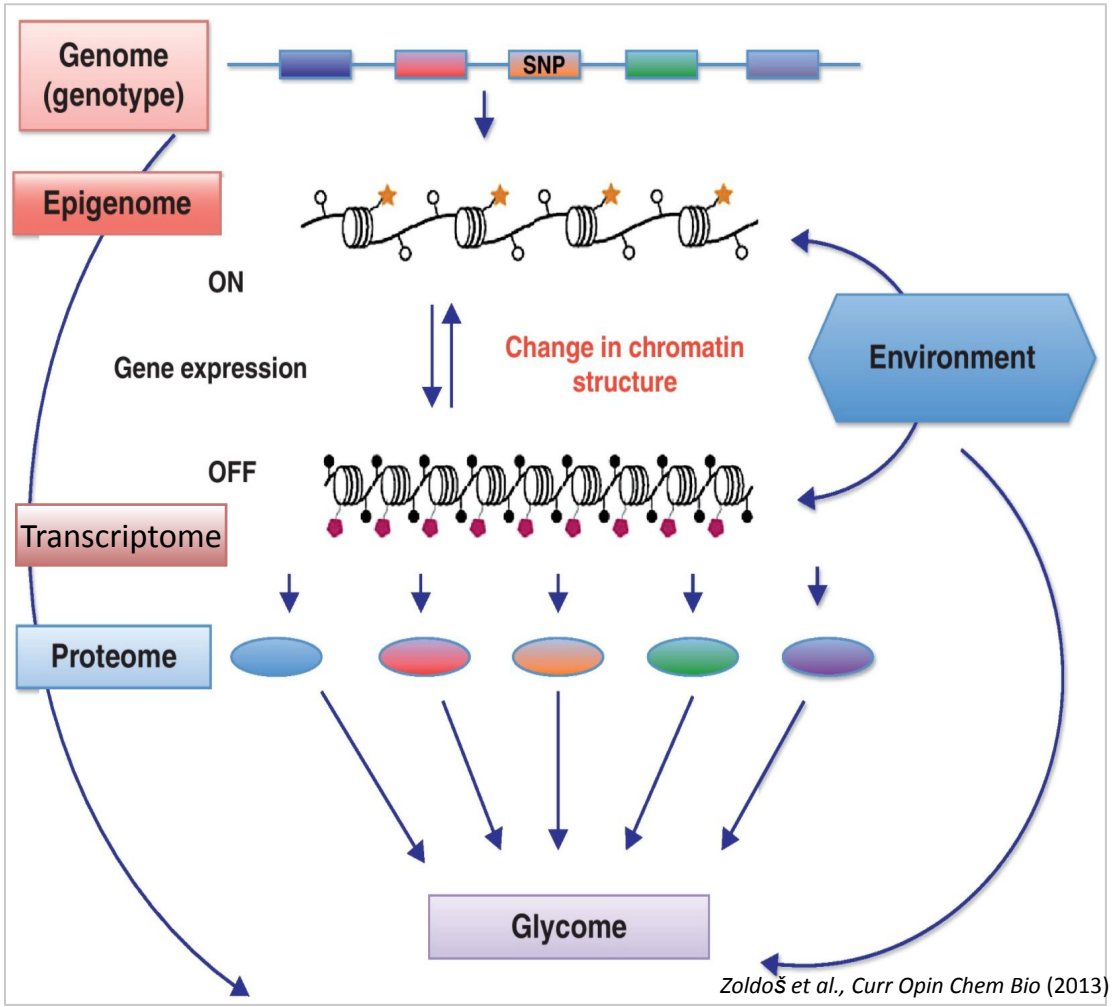
Understand:

- What glycomics is and where it fits in relation to other “omics” in clinical care
- Importance of glycans in human health and disease; examples of diseases where glycans play a critical role
- Importance of glycans in infections eg. influenza and SARS-Co-V-2
- What the endothelial glycocalyx is, and how to clinically assess and improve its function

Glycomics:

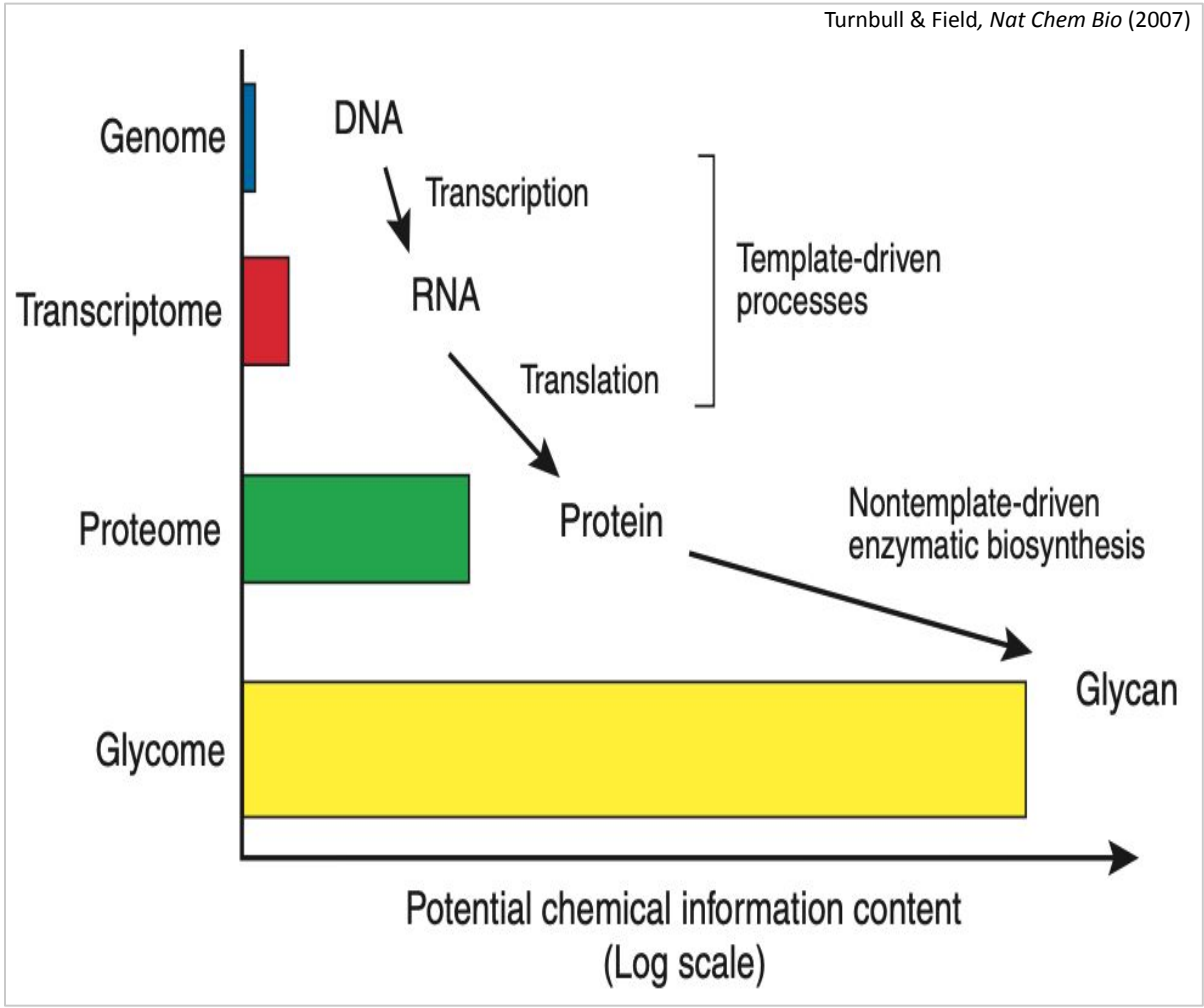
The study of the entire complement of sugars, both free and as part of complex molecules

Glycome: The main class of post-translational modifications

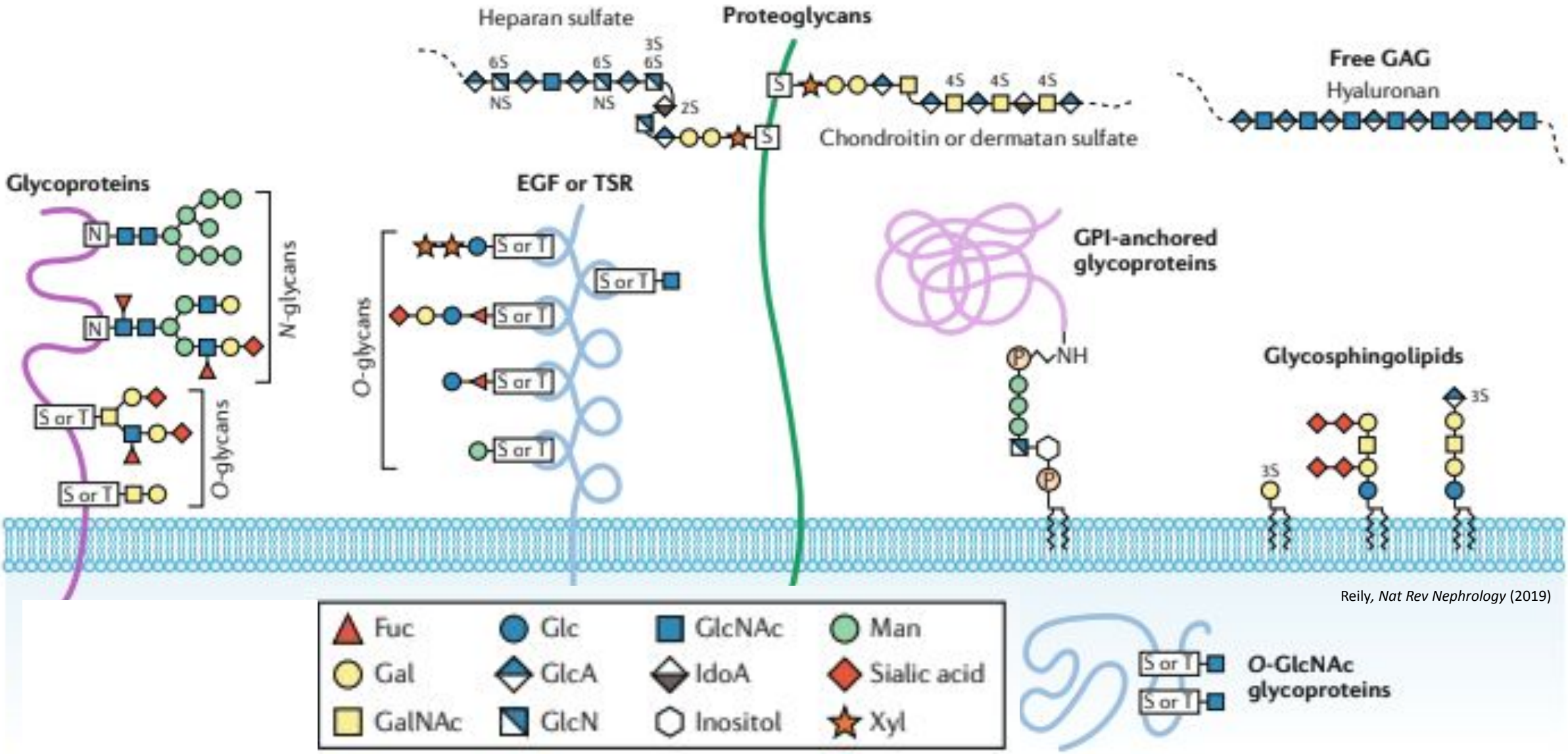


Zoldoš et al., *Curr Opin Chem Bio* (2013)

Turnbull & Field, *Nat Chem Bio* (2007)

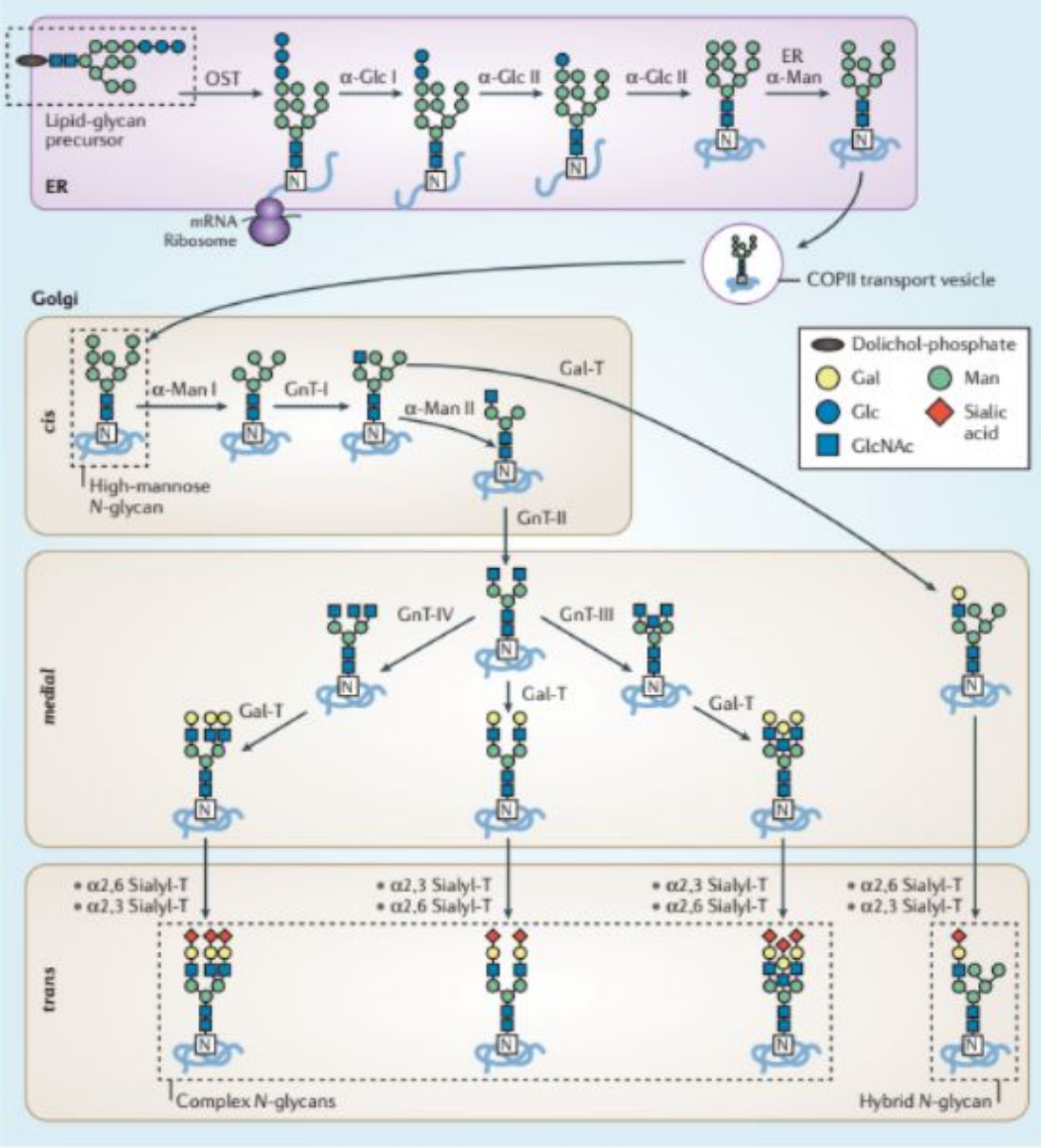


Glycans: Polymer chains of covalently linked monosaccharides
Main classes of human glycoconjugates.



Reily, *Nat Rev Nephrology* (2019)

Glycoproteins are synthesized in the ER and Golgi



Sequential concerted multi-step process thru ER and Golgi

Initiation: determines target proteins

Immediate core extension: options for different core structures

- Elongation / Branching: expands, repeats common structural motifs
- Capping: terminates oligosaccharide chains

Reily, *Nat Rev Nephrology* (2019)

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Glycosylation is highly conserved across mammalian cells

16 distinct pathways with ~200 glycosyltransferases:

- 2 types of lipid glycosylation
- 14 distinct types of protein glycosylation
- 11 types of O-glycosylation
- N-glycosylation
- C-mannosylation
- GPI anchor generation

Greatly amplifies the proteome by *producing diverse forms with myriad properties and functions*

~1% genes directly involved in glycosylation

~50% all proteins are glycosylated, majority of human nuclear and cytoplasmic

> 85% secretory proteins are glycosylated

Glycans have myriad roles and functions in health

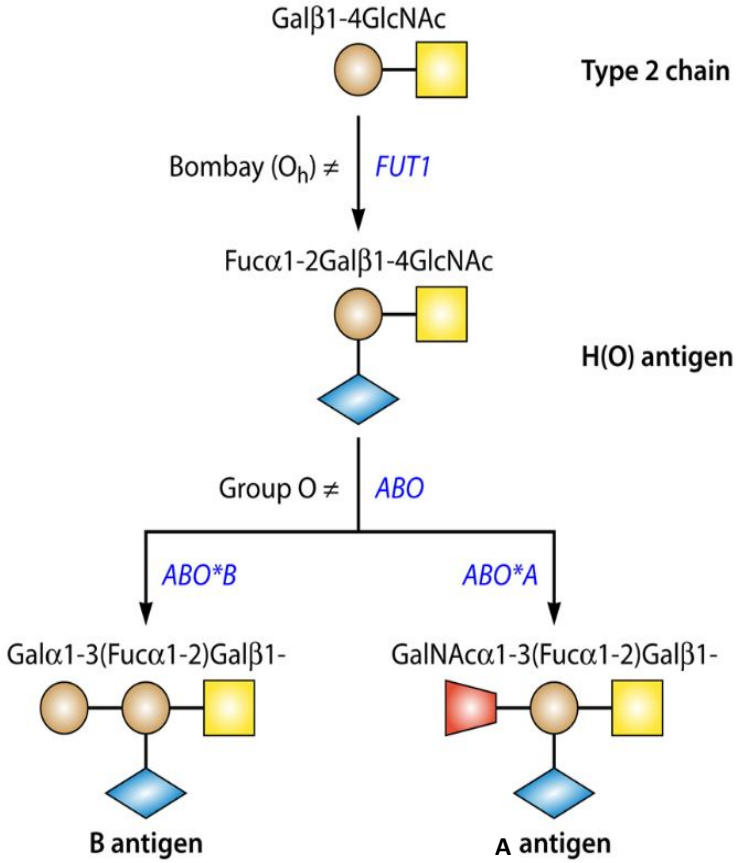
- Immune cell interactions are mediated by surface molecules that drive cellular activation via binding of membrane-bound glycoconjugates to sugar-specific receptors
- Endothelial cell-leukocyte interactions – crucial for trafficking – are controlled by glycan adhesion molecules also regulated by glycosylation
- Crucial and multifaceted roles in B cell and T cell differentiation
- Effector functions of antibodies
- Marking of apoptotic cells for clearance

Glycans have myriad roles and functions in disease

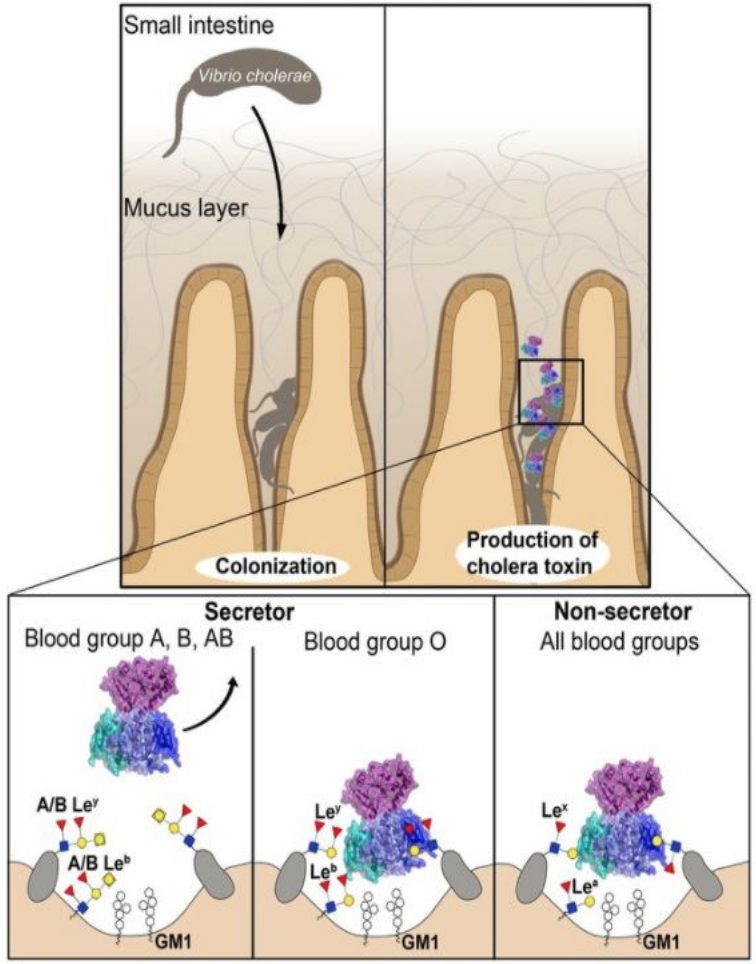
- Congenital disorders of glycosylation (CDGs)
- Host microbial recognition
- Immune evasion in pathogens and symbionts
- Pathogenesis of autoimmune disease, e.g. RA, IgA nephropathy, IBD
- Diabetes involves abnormal O-linked N-acetylglucosamine-mediated signaling, enhanced glycation of multiple proteins
- Influence metastatic properties, inhibition of apoptosis, resistance to chemotherapy

Histo Blood Group Antigens (HBGAs)

- First glycan antigenicity established
- Surface of RBCs, gut and respiratory epithelia, secretions
- Expression regulated by glucosyltransferase genes in ABO, FUT1-2 loci.
- Polymorphic in human populations
- Influence susceptibility to infection, inflammation



Cooling, Clin Micro Rev (2015)



Heggelund et al., *PLoS Path* (2016)

IN THE CLINIC

Blood group O more severe *V. cholerae* infection
Human norovirus selectivity for ABO(H) and Lewis antigens
Blood group O resistance to SARS-CoV-2 infection – early evidence

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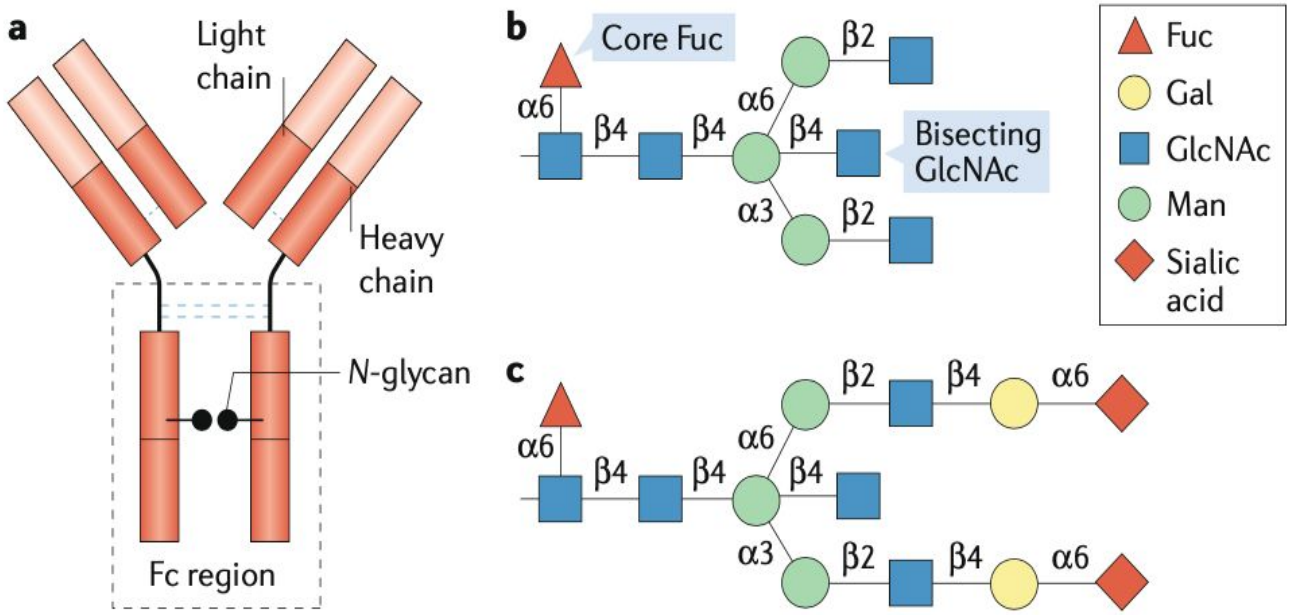
Glycans: N-linked

- Structural components, e.g. cell wall, ECM
- Modify protein properties, e.g. stability, solubility
- Direct trafficking of glycoproteins
- Mediate cell signaling, cell–cell and cell–matrix interactions

PRO-INFLAMMATORY



Reily et al., *Nat Rev Nephr* (2019)

ANTI-INFLAMMATORY

IMMUNE SYSTEM

- T and B cell differentiation
- Migration targeting
- Unique effector functions, altered Fc and other receptor affinities
- Self vs non self, pro- vs. anti-inflammatory

IN THE CLINIC

- Sialic acid- and gal-deficient IgG – RA, SLE, IBD, HIV, mycobacterial infections
- Pharmaceutical therapeutics – etanercept, infliximab rituximab
- Lack of different glycosylation in expression hosts leads to non-identical synthetic proteins >> *immunogenicity*
  e.g. Alpha-gal allergy, Neu5Gc

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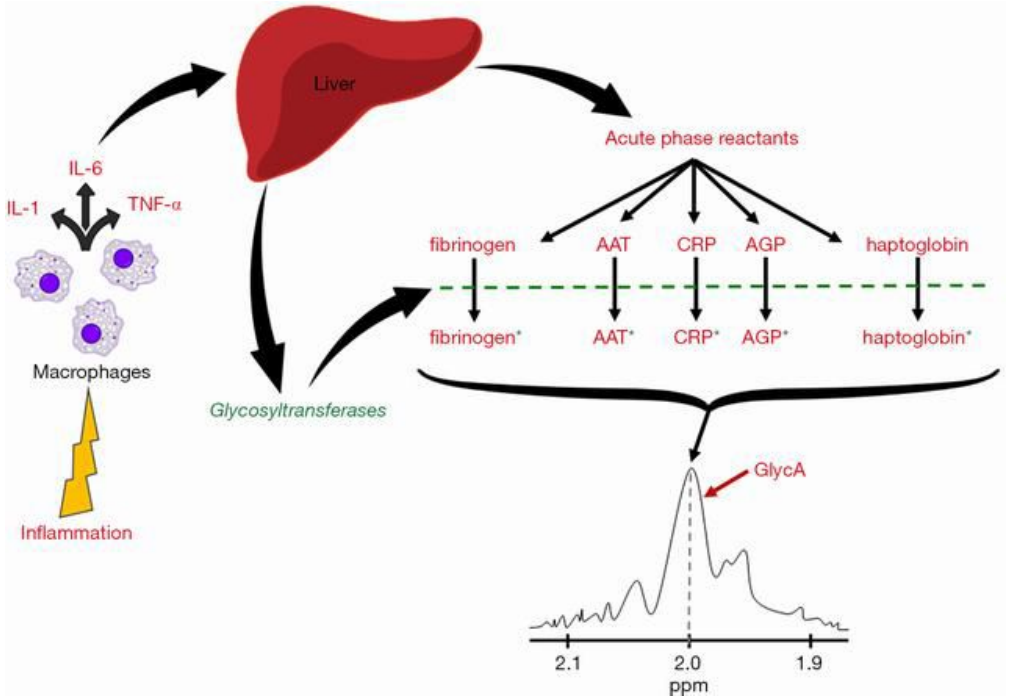
GlycA is a biomarker of inflammation

- Acute phase reactants are almost all N-linked glycoproteins.
- Hepatic glycosyltransferases activity evolves during the inflammatory process.
- Extension of glycan residues, increased branching, removal of sialic acid/galactose residues.
- Number and complexity of N-glycan side chains attached to acute phase reactant proteins
- Elevated in acute and chronic inflammation
- CVD, RA, IBD, psoriasis, lupus, predicts death



Ballout and Remaley, *JLPM* (2020)

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Glycans: O-linked

Extracellular, secreted glycoproteins

Mucins: many O-linked glycans, extended cores create highly protective gel-like substance (mucus)

IMMUNE SYSTEM

Protection from infection and self-recognition

B- and T- cell surfaces, modulated for cellular differentiation, activation, e.g., CD43, CD45

Regulate T cell functions: migration, receptor signaling, survival, apoptosis

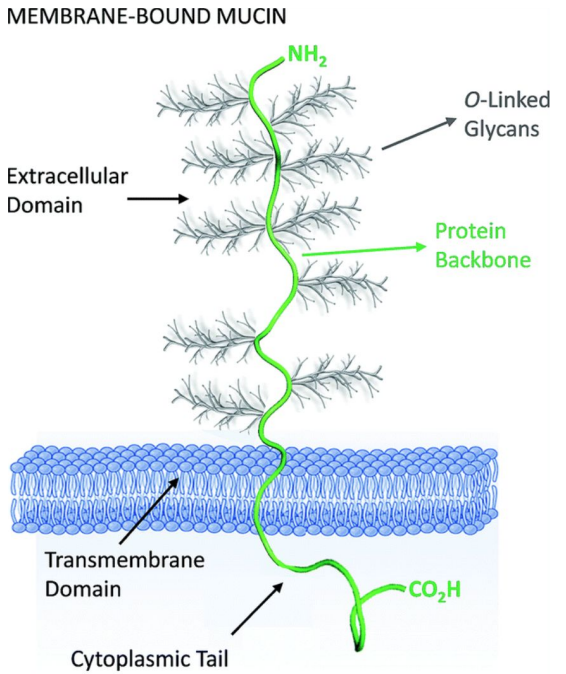
IN THE CLINIC

Infected burn wounds

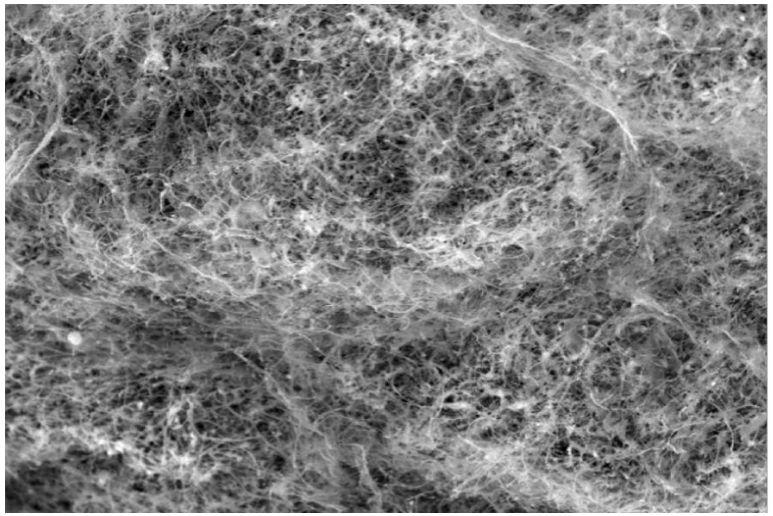
Gal-deficient IgA1 nephropathy

Diabetes, cellular response to insulin

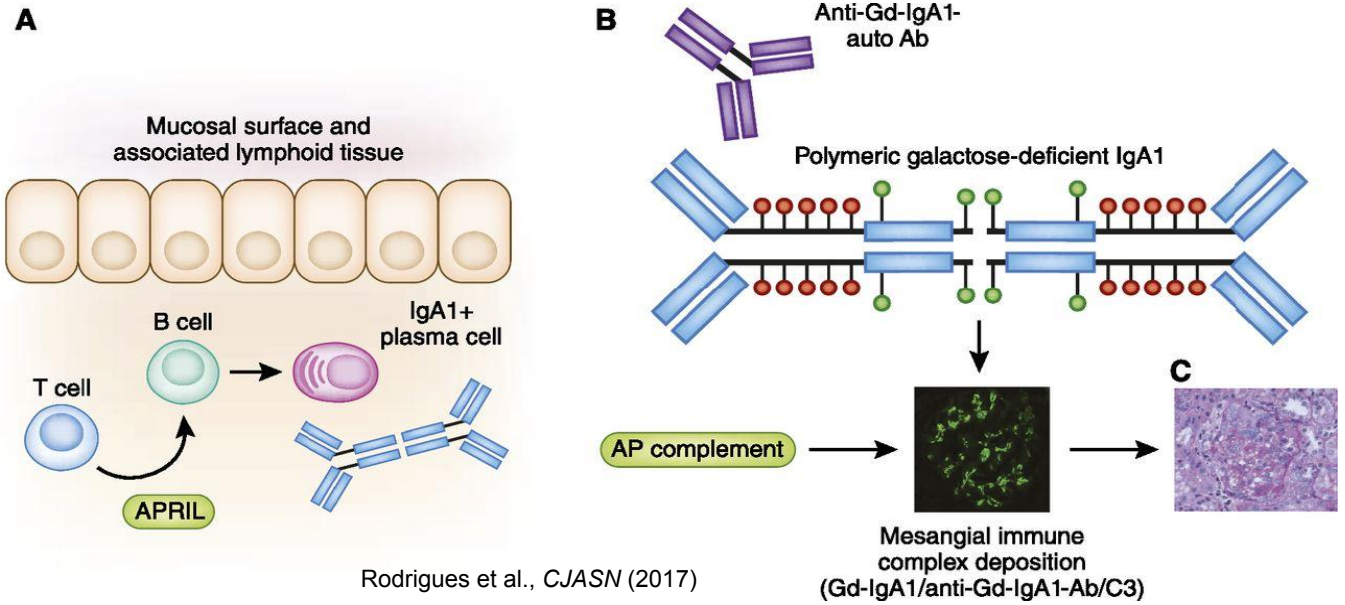
Cancer cell immune modulation



Martínez-Sáez et al., *Chem Soc Rev* (2019)



Wheeler et al., *MIT News* (2019)



Rodrigues et al., *CJASN* (2017)

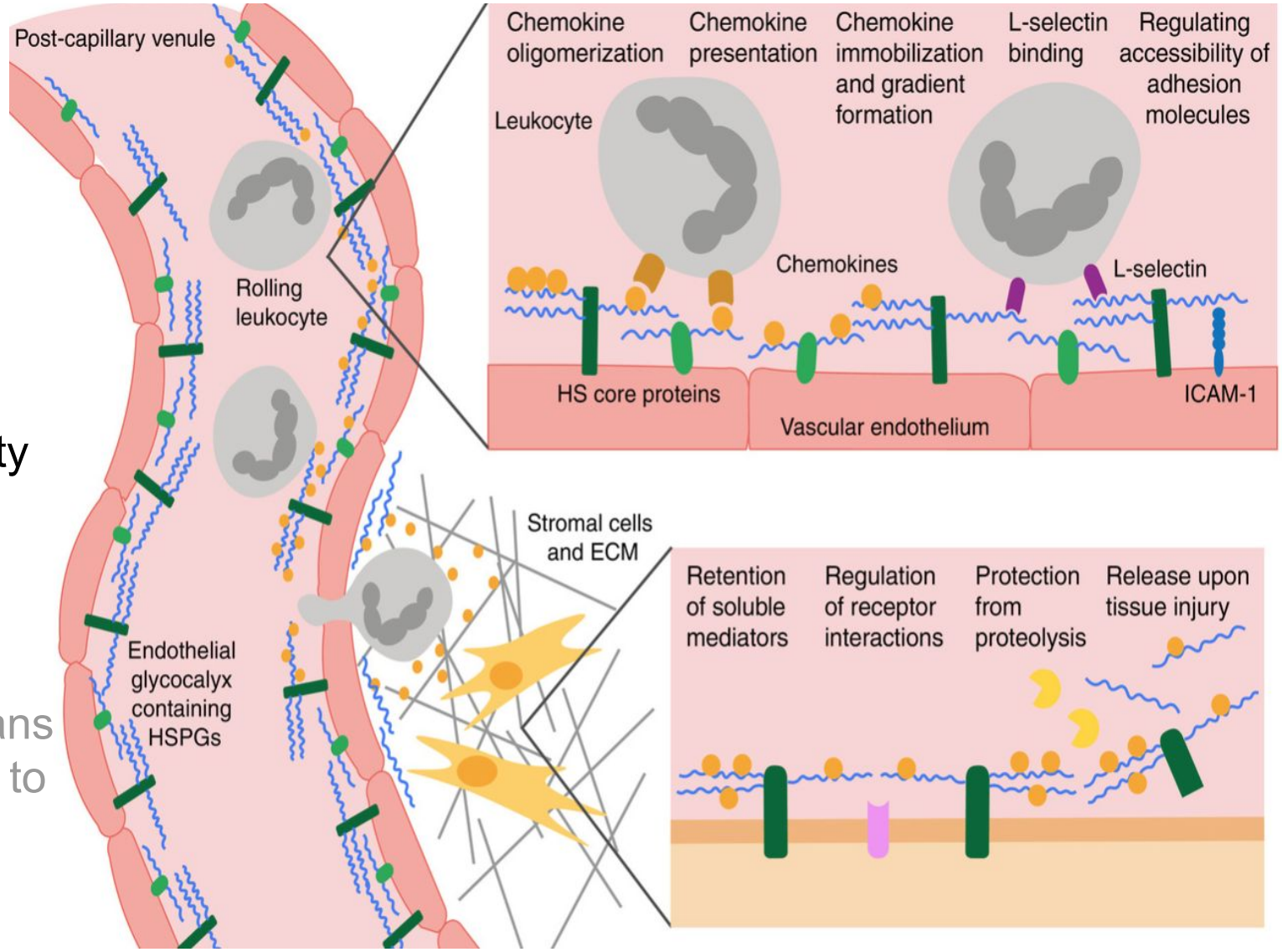
Glycans: Other classes

Glycosaminoglycans (GAGs)

- Extended, negatively charged, high water content, functionally diverse sugar chains
- Large portion of proteoglycan molecular mass
- Crucial to GCX formation, integrity

Proteoglycans

- ECM glycoproteins
- Contain canonical N-and O-glycans
- Long sugar repeats (GAGs) bind to O-linked motifs
- Inflammation, infection, cancer metastasis.



Collins & Troeberg, *J Leuk Bio* (2018)

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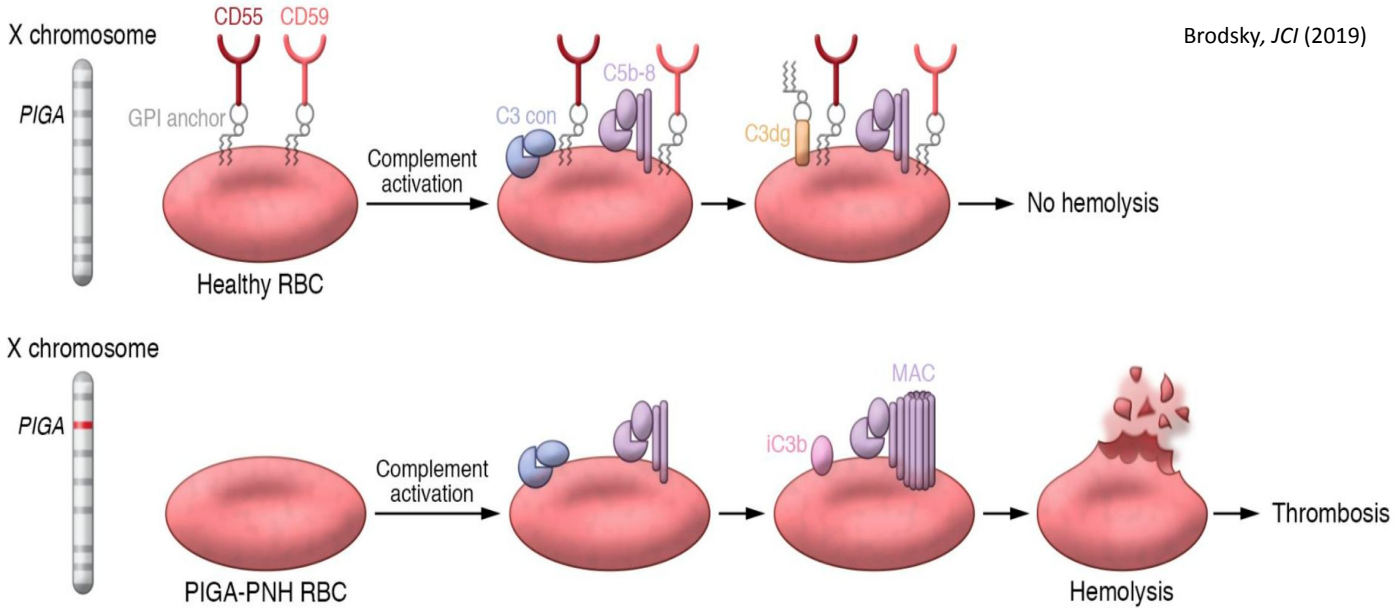
Glycans: Other classes

GPI anchors

- Complex cell surface molecules with a common core
- Apical side of polarized cells
- Highly dependent on proper Golgi sorting

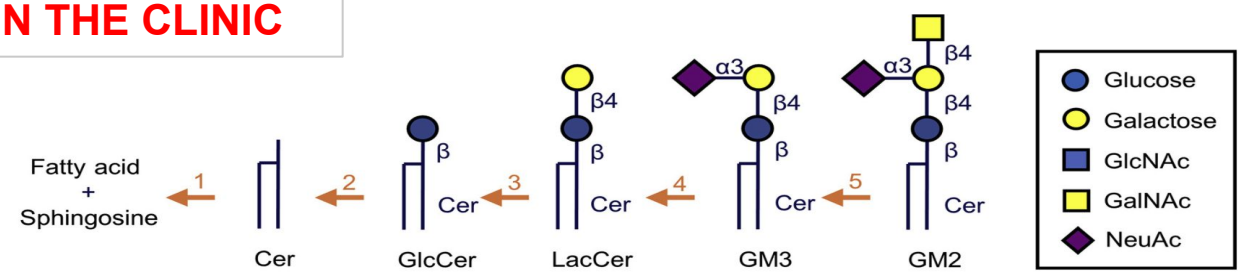


Brodsky, *JCI* (2019)

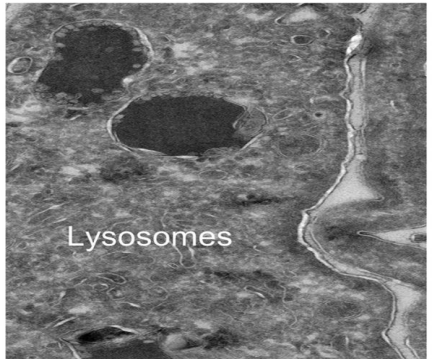
IN THE CLINIC

Glycosphingolipids (GSLs)

- Sphingolipid with glycan attached to ceramide
- Among most abundant glycolipids in humans
- Cell membrane lipid bilayer



Aerts et al., *Cur Opin Chem Bio* (2019)



Enzyme	Gene	Disease	Primary storage GSL
1: Acid ceramidase	Asah	Farber disease	Cer
2: Glucocerebrosidase	Gba1	Gaucher disease	GlcCer
5: β-Hexosaminidase	HexA/B	Tay-Sachs /Sandhoff disease	GM2

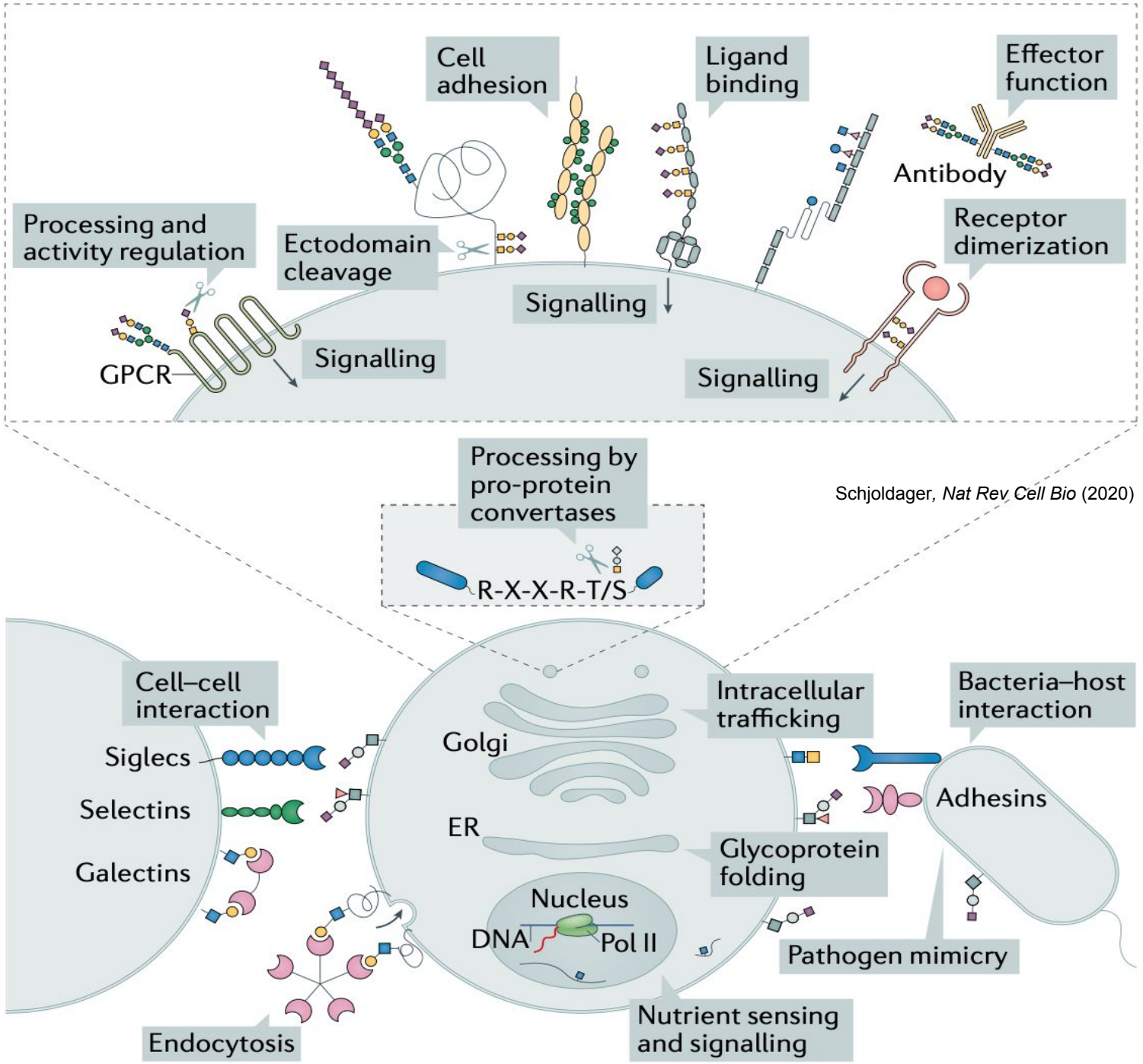
Glycosylation Disorders

IN THE CLINIC

CDGs: often embryonic lethal, underlying vital role of glycans; multisystemic, wide spectrum and clinical severity

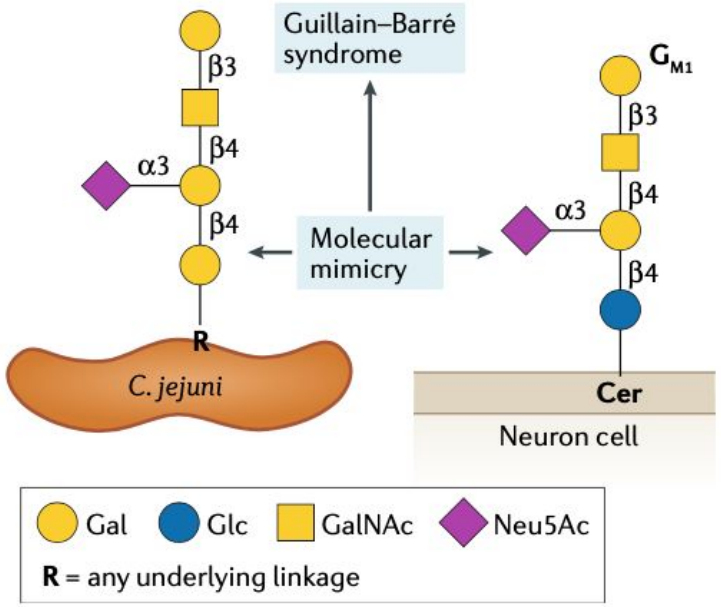
Type I: abnormalities in formation of oligosaccharide structure on glycolipid precursors before attachment (ER)

Type II: defects in control of N-linked branching structure on nascent glycoproteins (Golgi)



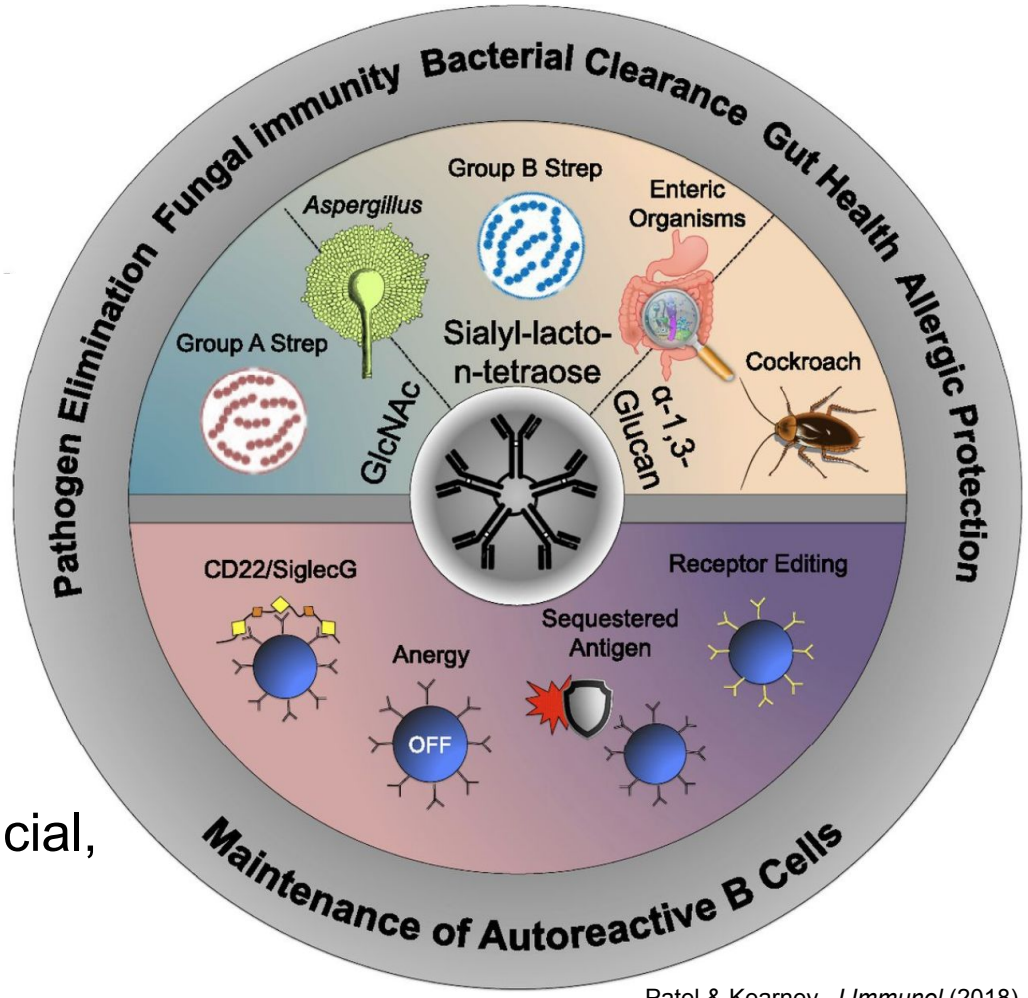
Glycans: Microbial

- Bacterial LPS, gram (-) PGN, teichoic acids, viral capsules, fungal mannans
- Display host-like glycans for immune evasion, including symbionts, e.g. gut microbiome
- Glycosylation enzymes can modify host proteins for benefit, e.g. carbon source, cell adhesion



Poole et al., *Nat Rev Micro* (2018)

- Host Abs to shared glycans can be beneficial, e.g. suppress allergy



Patel & Kearney, *J Immunol* (2018)

IN THE CLINIC

Toxins – cholera, botox, STEC, cytolethal distending toxin B

Vaccines – pneumococcal, MCC

Pathogen-host glycan similarity can be associated with risk of autoimmunity via molecular mimicry

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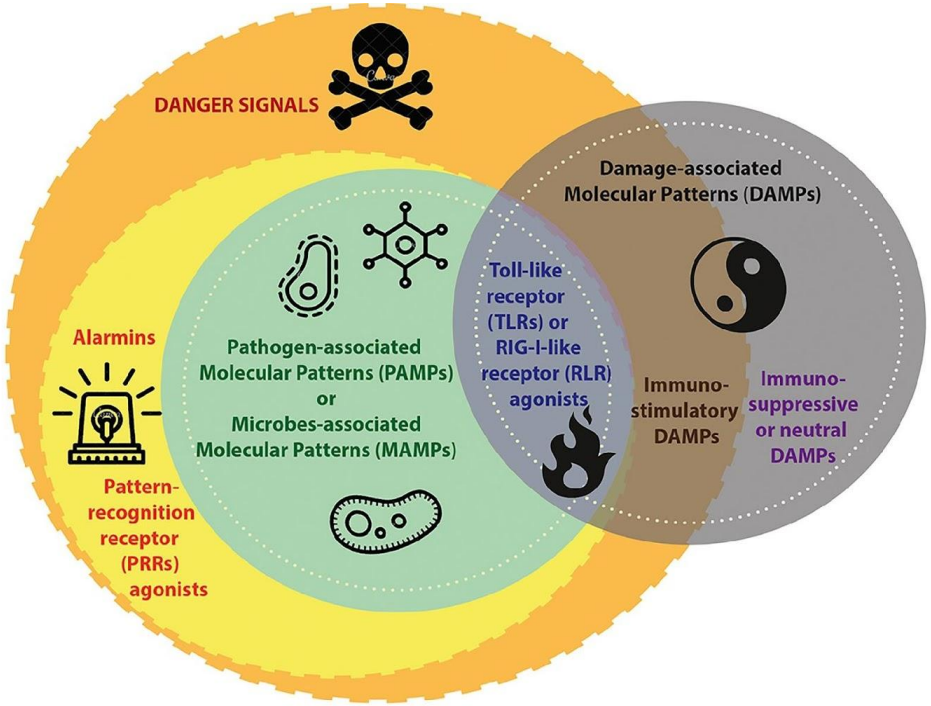
Glycans: Molecular pattern ligands and receptors

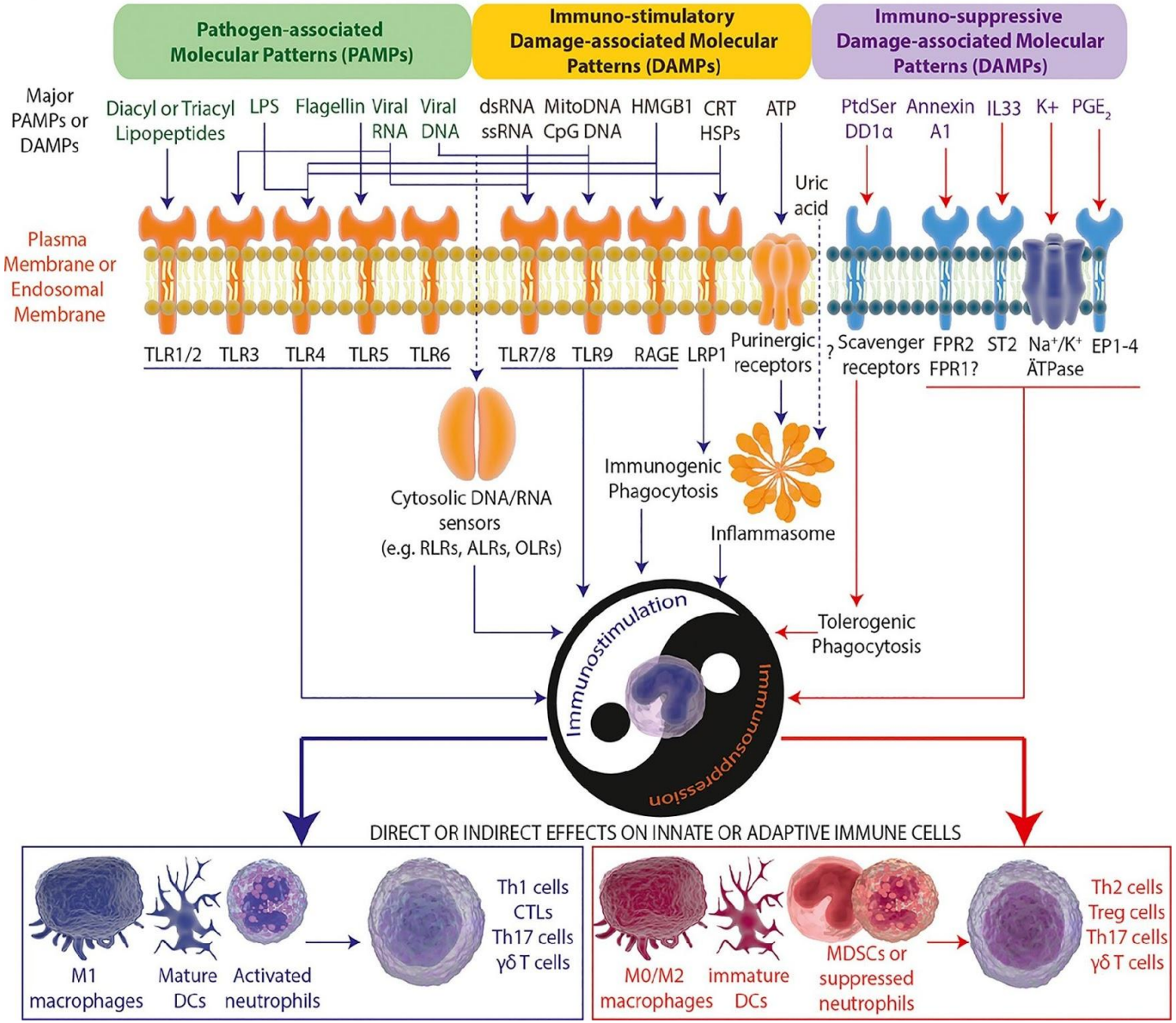
Integration of these signals is critical to guide immune system discernment

- DAMP's, PAMP's, SAMP's

  Core inflammatory pathways, e.g. IBD, sepsis, cancer, chronic disease in aging

  Promising immunotherapeutic targets

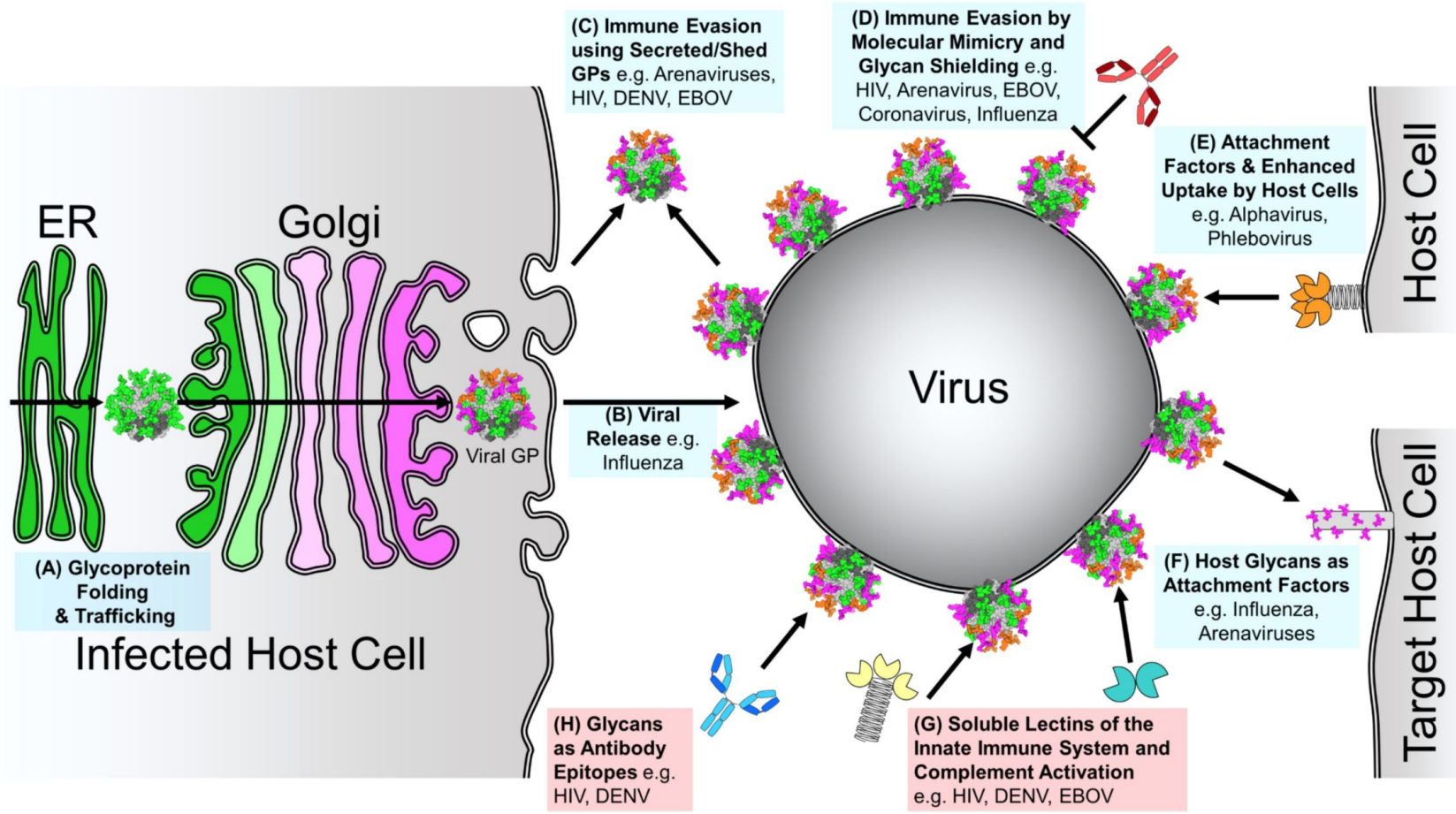




Garg & Agostinis, *Immuno Rev* (2017)

Glycans: Viral

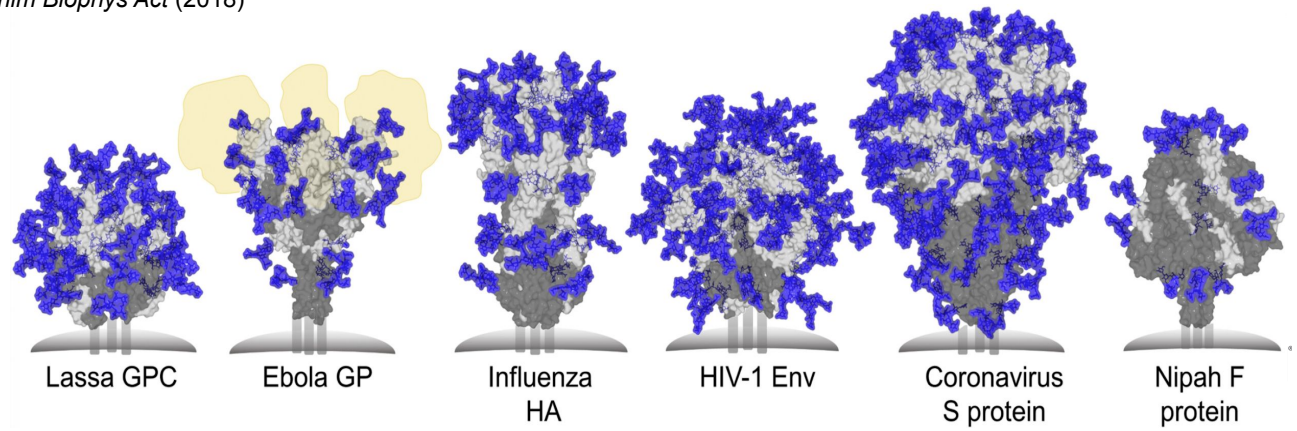
- Viral envelope shielding with host-derived glycans for immune evasion
- Viral glycan shedding for immune evasion
- Interference with host glycosylation
- Attachment factors



Watanabe, *Biochim Biophys Act* (2018)

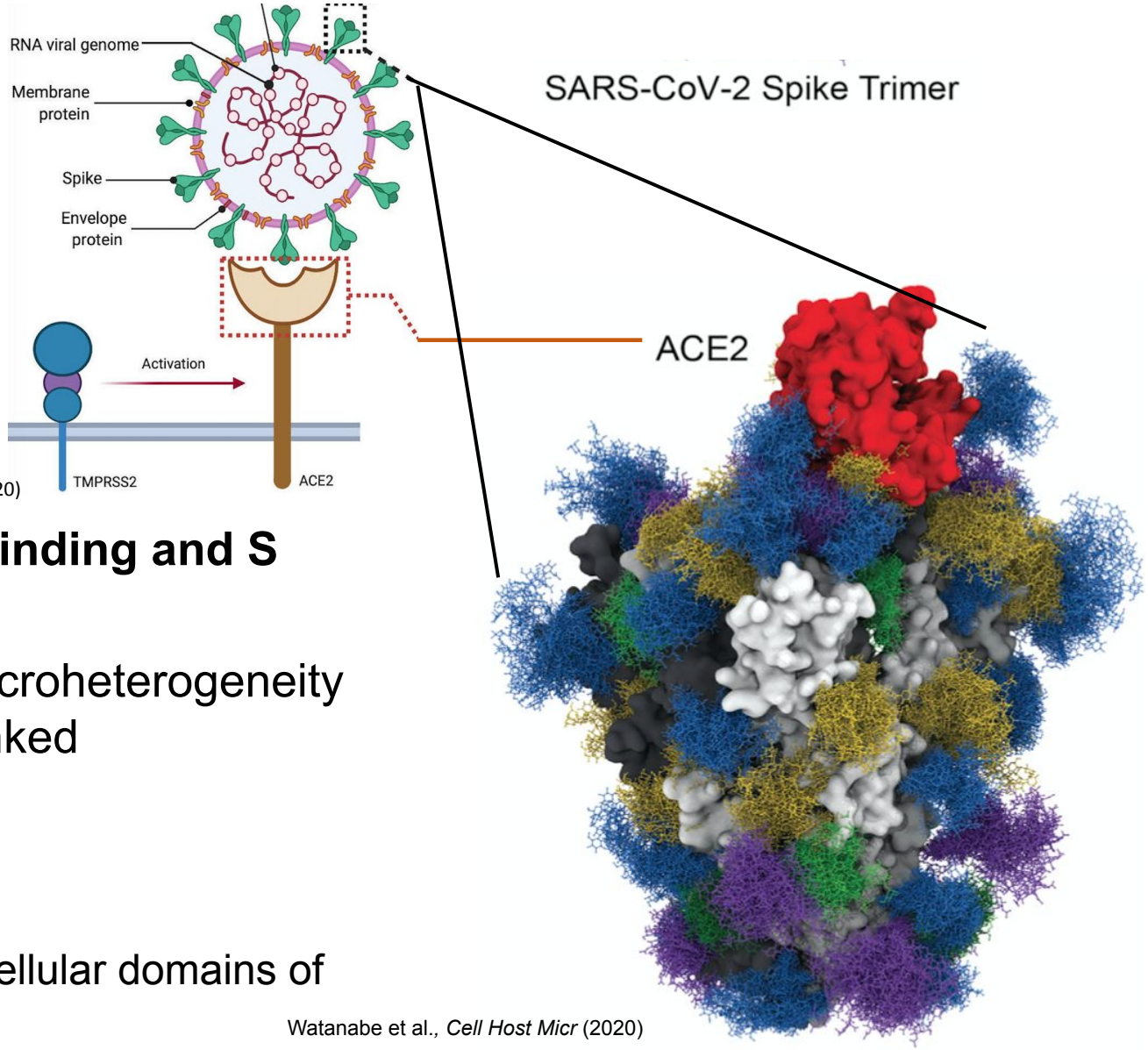
IN THE CLINIC

Vaccines: influenza, HIV, MERS
Neuraminidase inhibitors
Neutralizing antibodies



Important role of glycans on SARS-CoV-2 Spike protein

- Multiple MS-based approaches – glycomic and glycoproteomic
- Static and dynamic models of S alone + glycosylated soluble ACE2 complex
- Simulations of glycosylated S-ACE2 interaction



Wiese et al., *J Clin Path* (2020)

Glycans are essential in receptor-viral binding and S shielding

22 sites of S show site-specific N-linked microheterogeneity
6 sites of human ACE2 receptor show N-linked microheterogeneity

IN THE CLINIC

Strategic vaccine design
Competitive inhibition with decoy soluble extracellular domains of ACE2 as decoy

Watanabe et al., *Cell Host Micr* (2020)

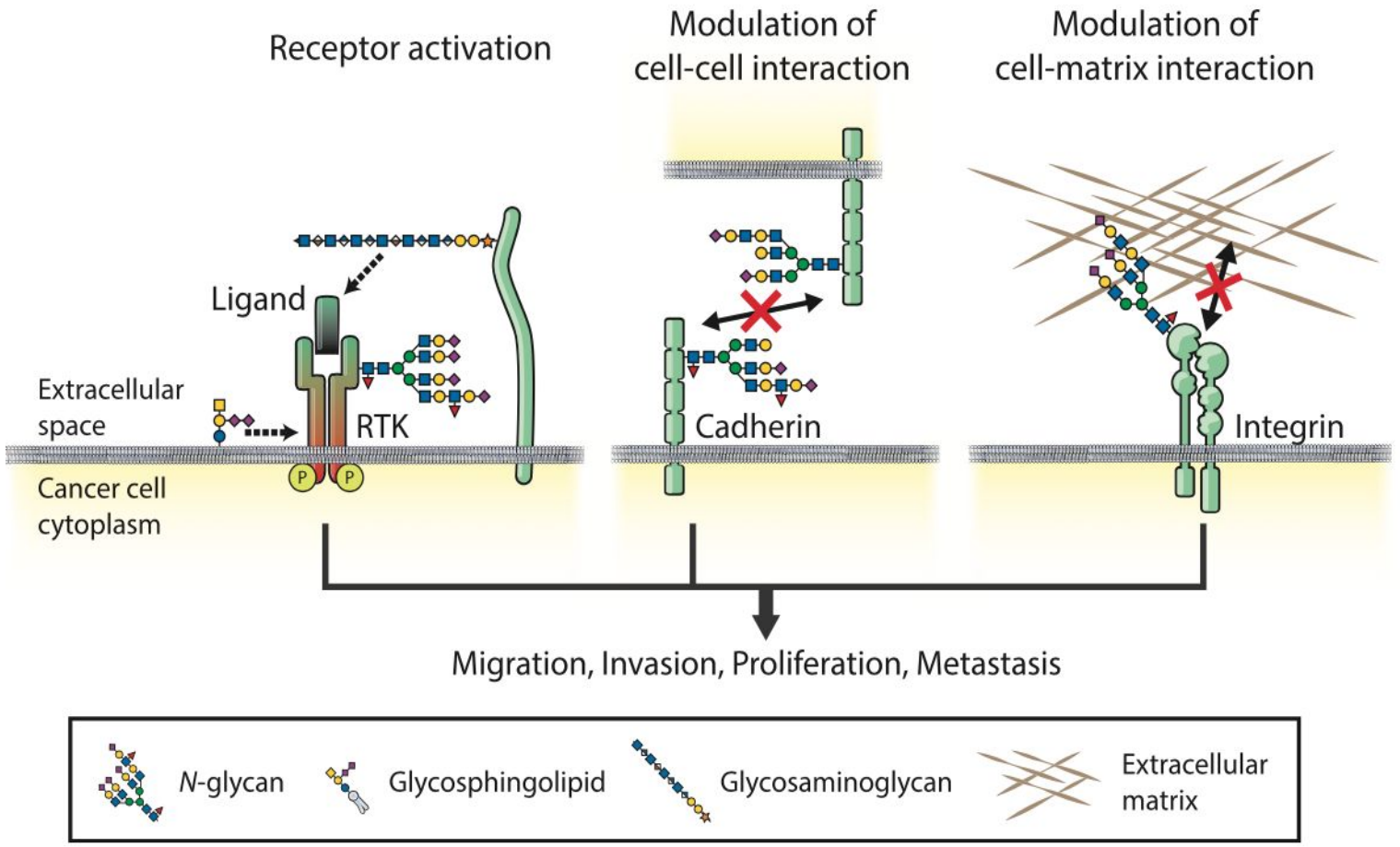
“Glycosylation is associated with cancer hallmark capabilities”

Migration, invasion, proliferation, and metastasis:

Receptor tyrosine kinases (RTKs) activated by altered receptor glycosylation, gangliosides, GAGs

Increased N-glycan branching on E-cadherin impairs cell-cell adhesion, downstream signaling

Glycosylation modulates tumor micro-environment, cell-ECM interactions; facilitates integrin-dependent signaling >>> cell growth and survival



Mereiter et al., *Cell Cancer* (2019)

IN THE CLINIC

Altered glycosylation is a near-universal feature of tumor cells

Promising targets for many biomarker and therapeutic applications

Glycan-based cancer markers

Blood:

CA15-3 (MUC1), CA19-9, CA125 (MUC16), CEA, PSA, AFP-L3

Limited sensitivity, specificity; used for prognostics, monitoring

Extracellular vesicles (EVs)

Circulating tumour cells (CTCs)

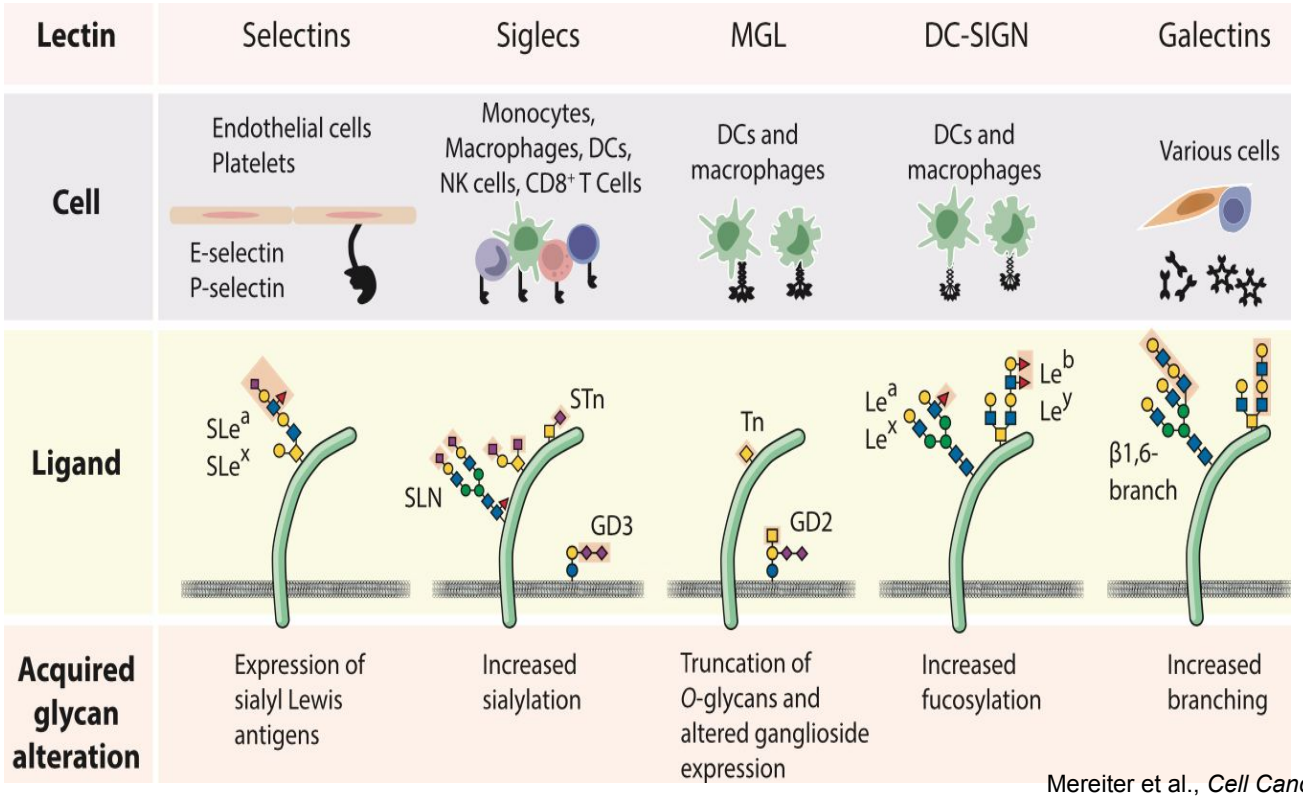
Tissue:

MALDI-IMS imaging

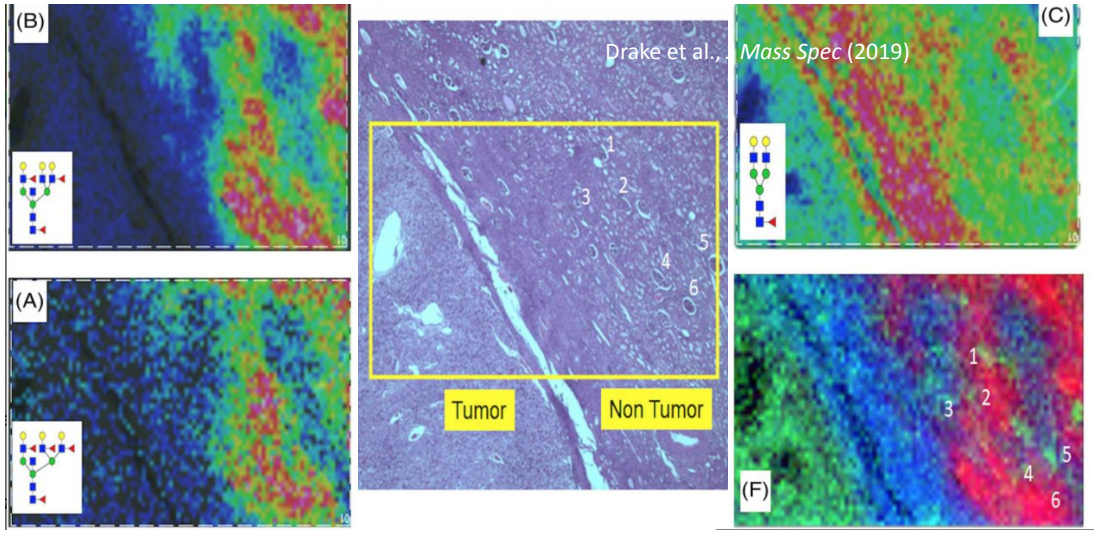
Differentiation, margins

IN THE CLINIC

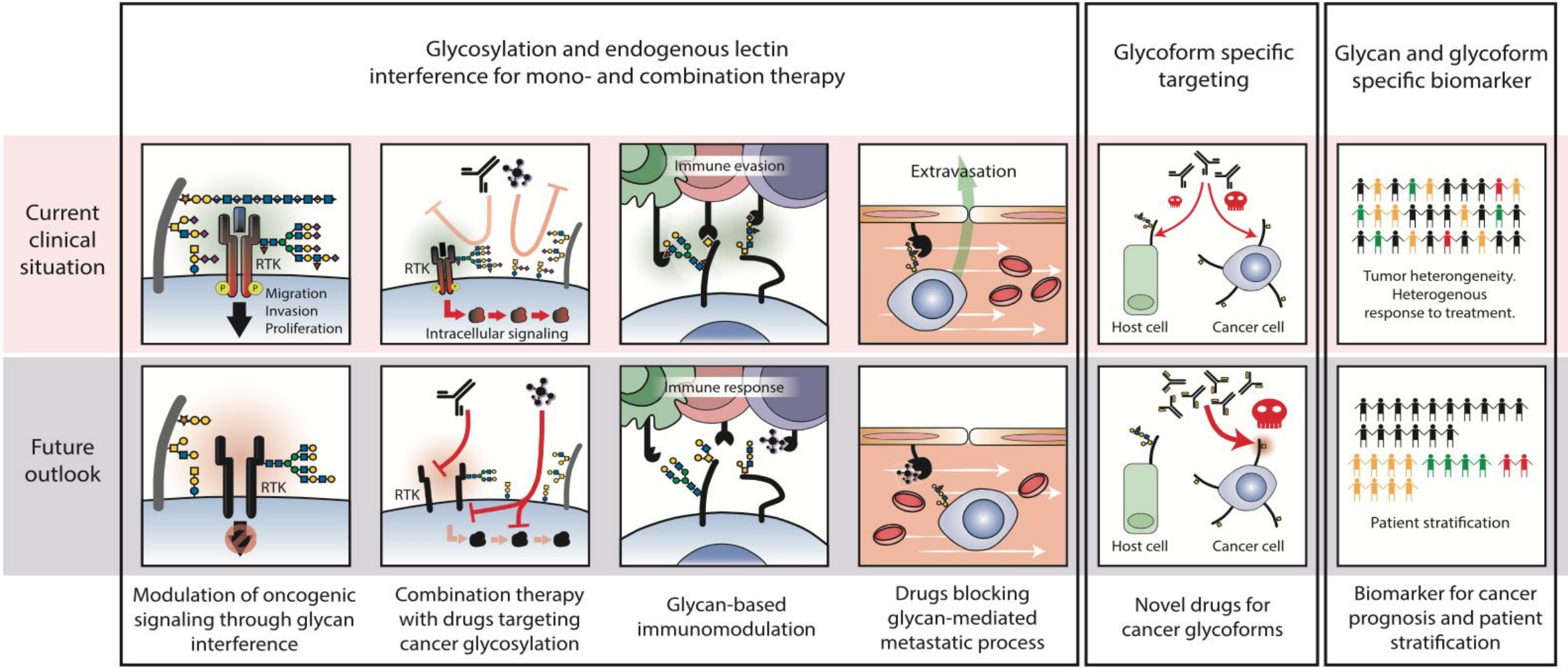
Breast, Ovarian, Prostate –sialic acid, Hepatocellular, Pancreatic, Colorectal



Mereiter et al., *Cell Cancer* (2019)



Glycosylation modulation for precision cancer therapeutics



Mereiter et al., *Cell Cancer* (2019)

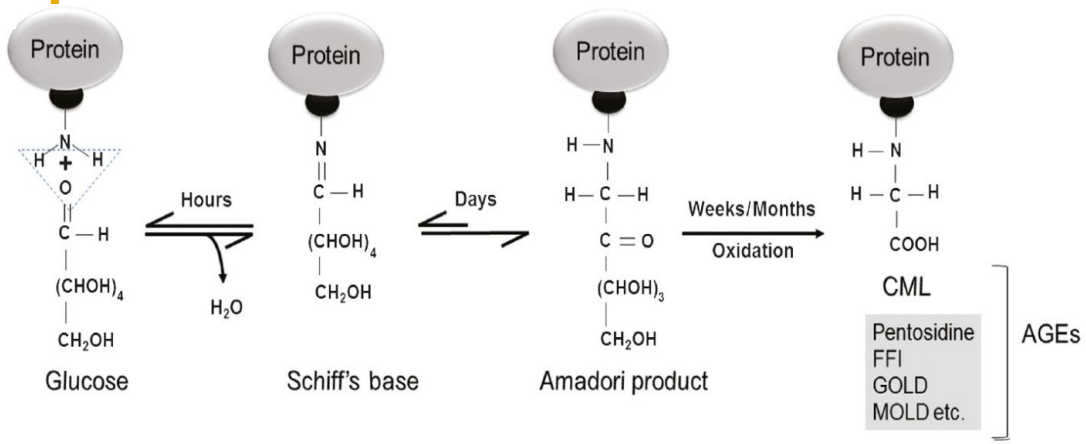
Glycation and the AGE-RAGE axis

Glycation: non-enzymatic, often multi-step reactions between reducing sugars, e.g. glucose, and proteins, lipids, or nucleic acids >>>

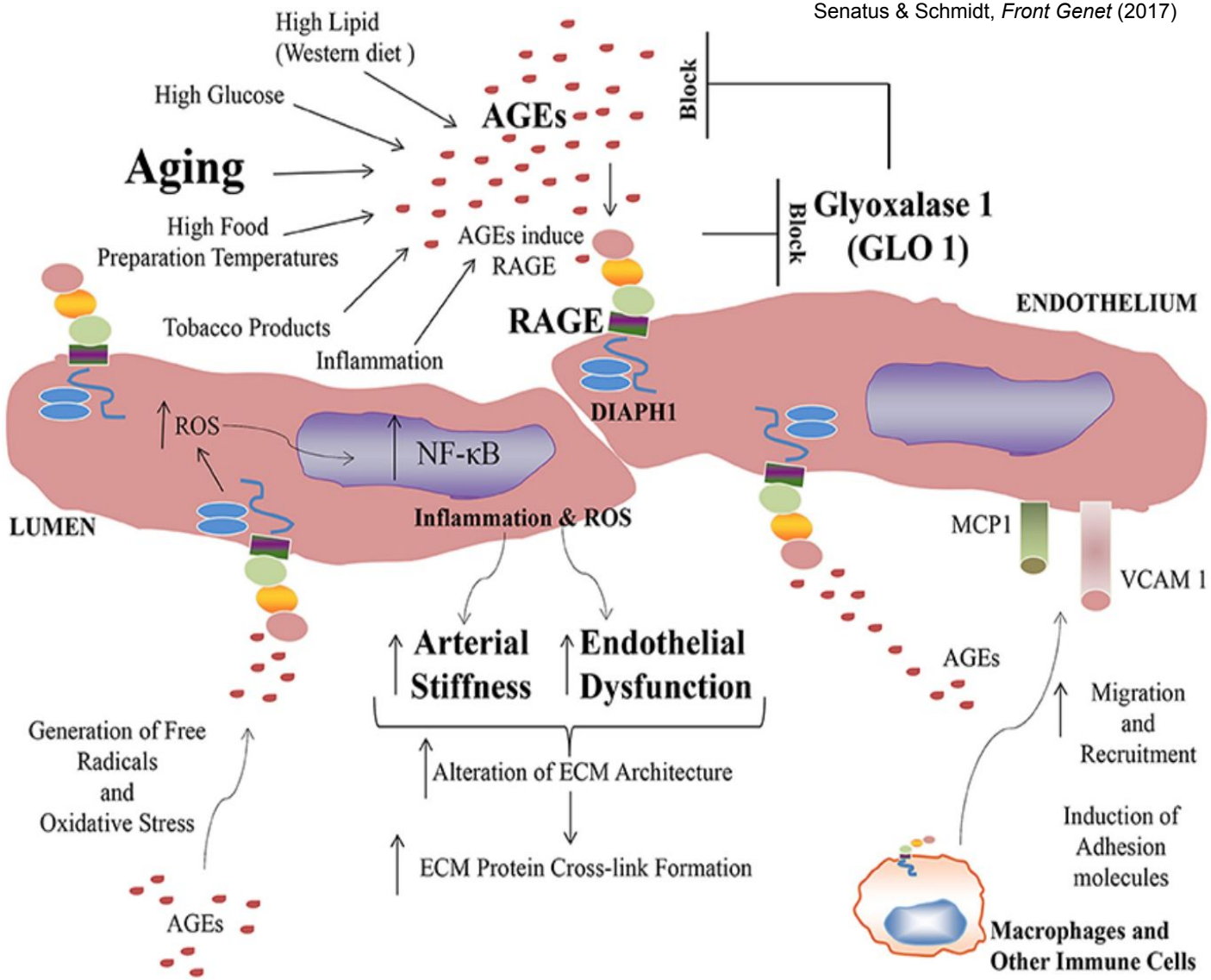
Advanced glycation end products (AGEs)
aka glycotoxins

HbA1C = Amadori product of hemoglobin and glucose

Pasupulati et al., *Biomol Con* (2016)



Senatus & Schmidt, *Front Genet* (2017)



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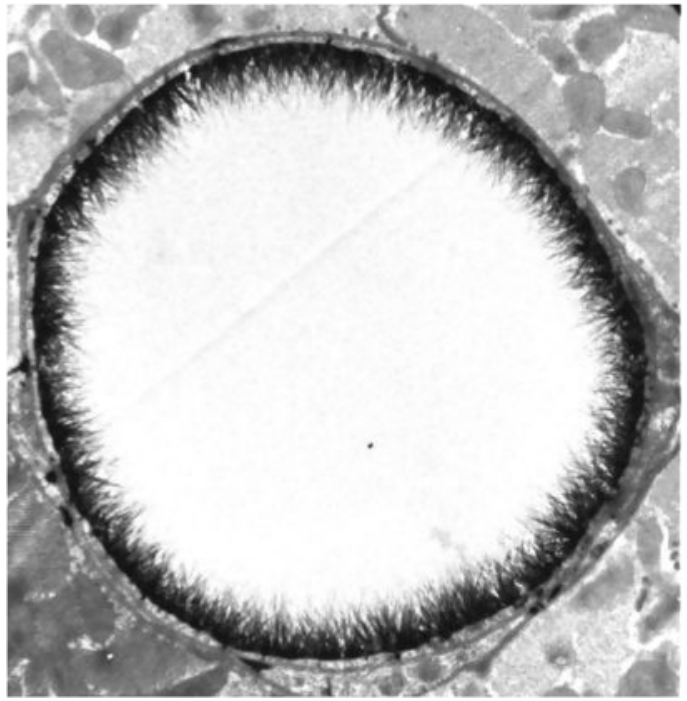
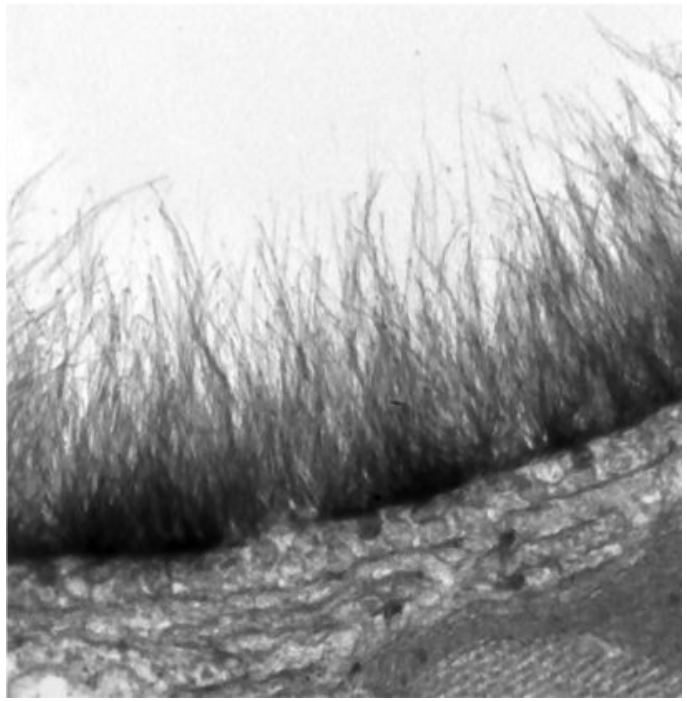
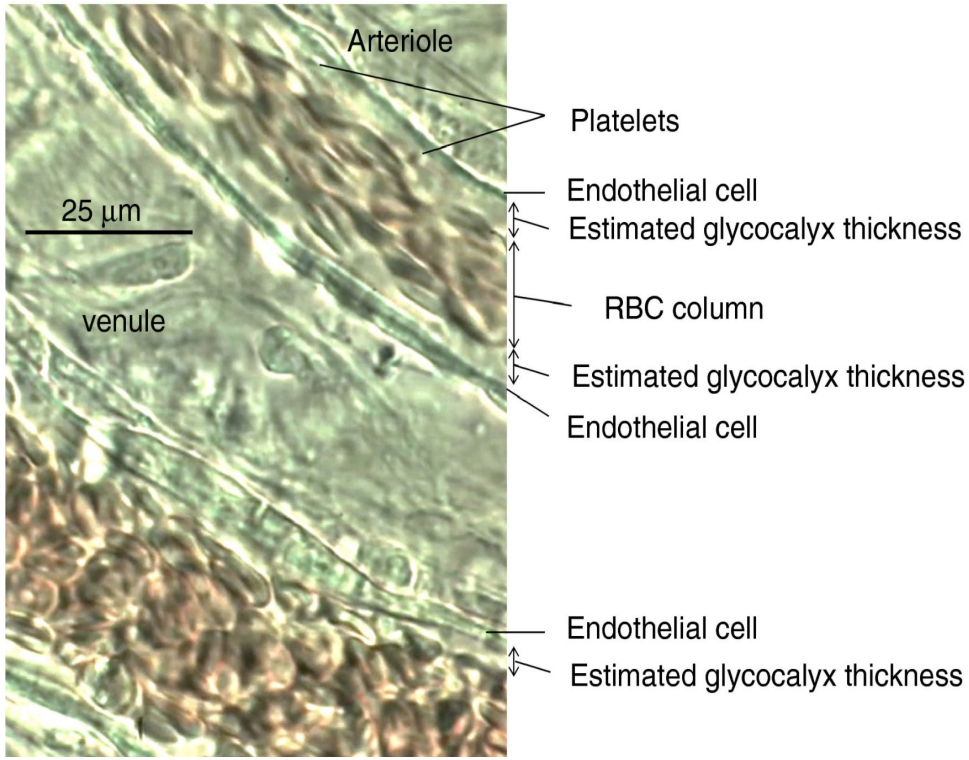
Intravascular imaging reveals the endothelial glycocalyx (EGX)

A microscopically thin gel-like layer of glycoproteins, proteoglycans, and GAGs coating the luminal side of the vascular endothelium







Iba & Levy, *J Thromb Haamost* (2019)

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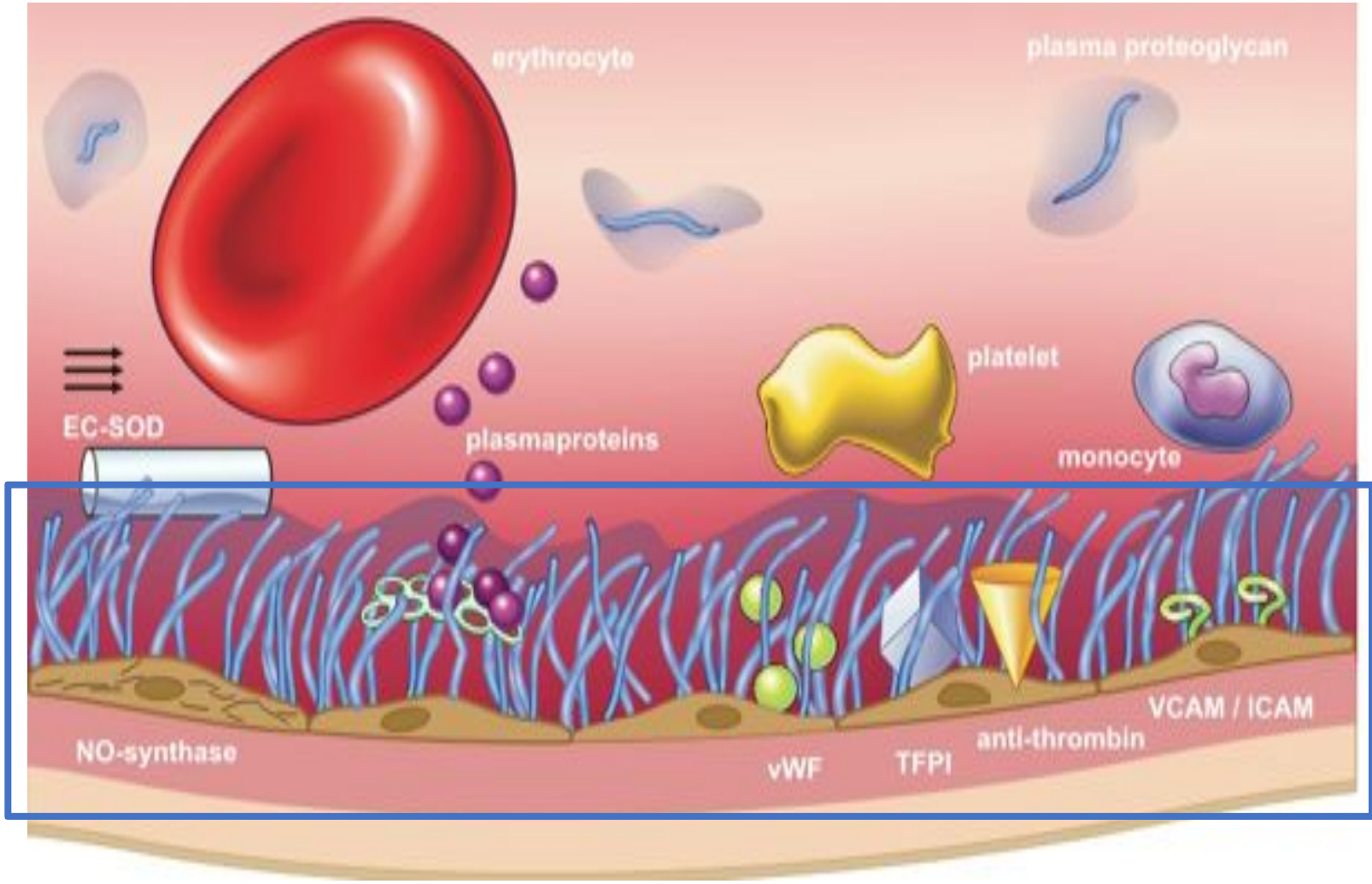
Roles of the endothelial glycocalyx

- Regulates permeability as a selective sieving barrier
- Helps regulate surface inflammatory response
- Arterial anti-adhesive
- Harbors coagulation regulatory factors
- Houses SOD – potent anti-inflammatory antioxidant
- Triggers production of nitric oxide (NO)



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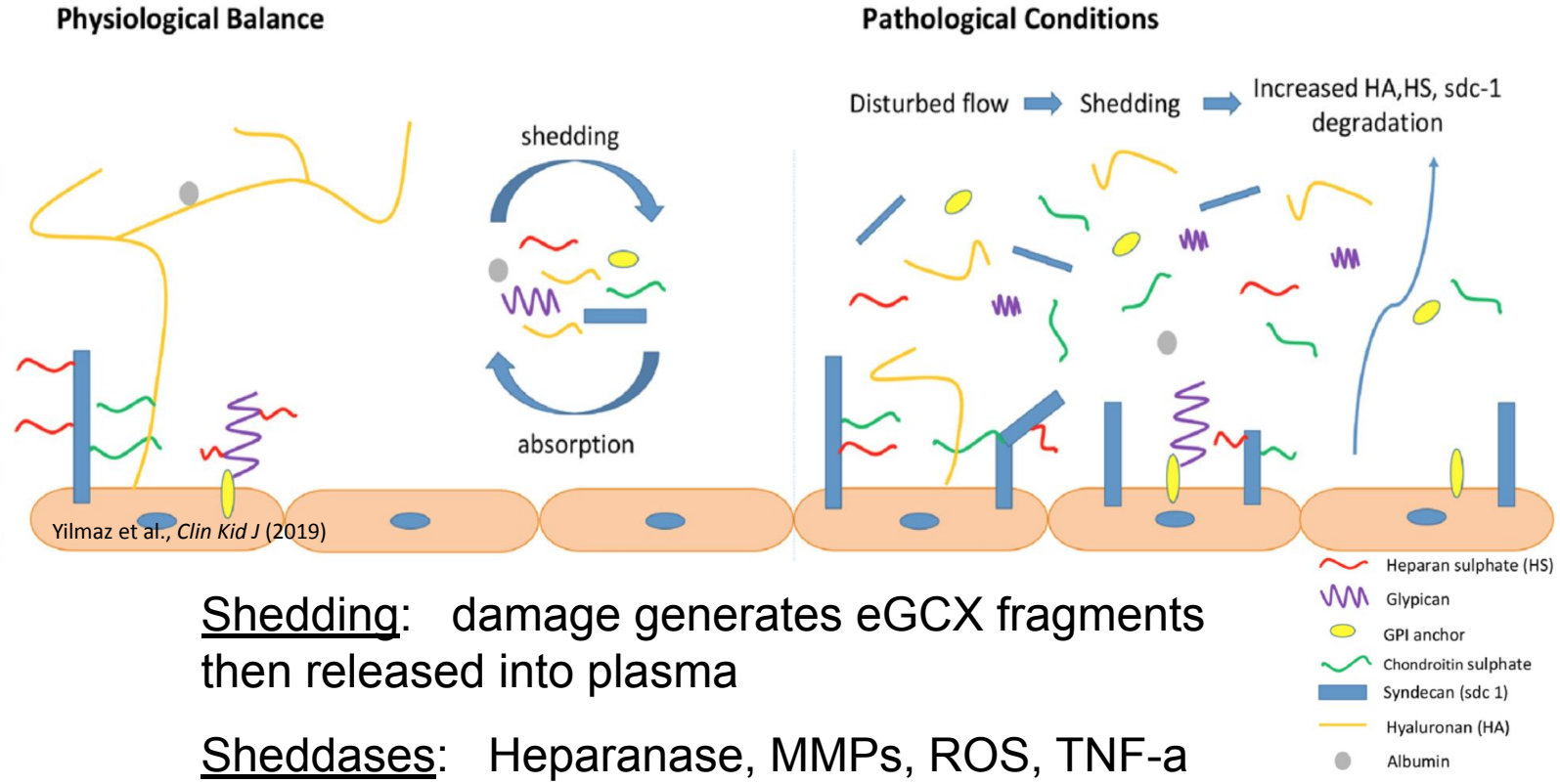
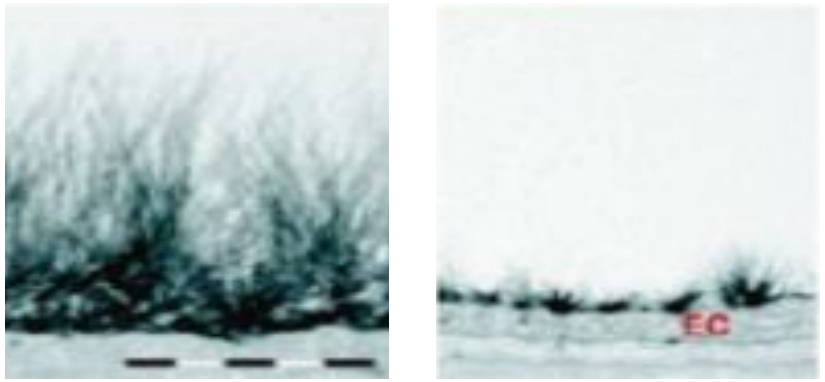
Healthy EGX turnover is a balance of shedding and synthesis



Yilmaz et al., *Clin Kid J* (2019)



Gouverneur et al., *J Int Med* (2006)

Shedding: damage generates eGCX fragments then released into plasma

Sheddases: Heparanase, MMPs, ROS, TNF-a

IN THE CLINIC

- Systemic or local inflammation
- Sepsis (TNF-a induced)
- Hyperglycemia, DM
- Ischemia-reperfusion (I-R) injury
- Renal disease
- Atherosclerosis

Unhealthy eGCX:

- Increased leukocyte and platelet adhesion, thrombin generation
- Increased macromolecule leakage, oxidative stress
- Reduced NO production

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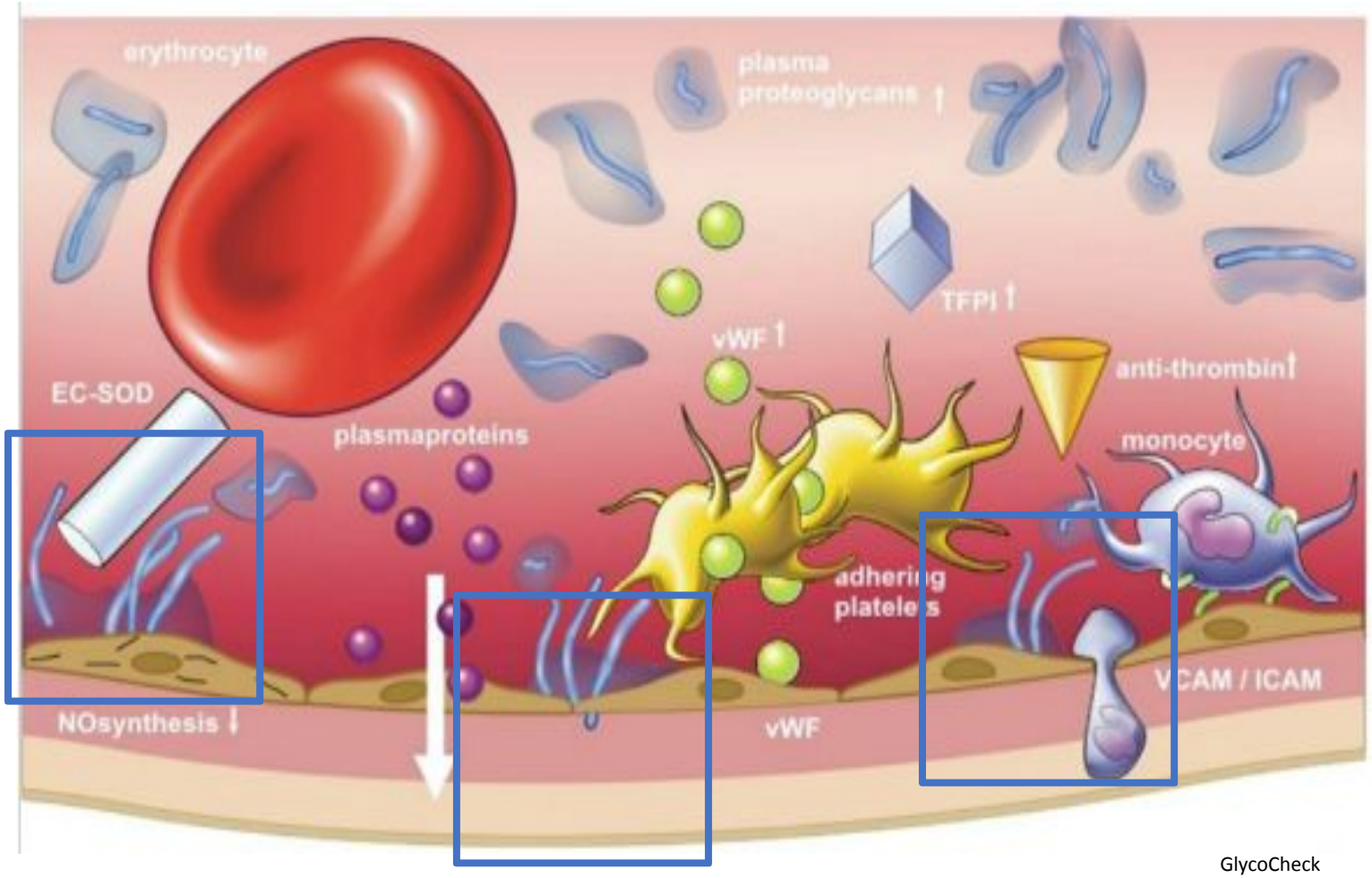
Endothelial glycocalyx: A proxy for endothelial health

Causes of EGX degradation:

High blood glucose, insulin
Inflammation
Oxidative stress
Toxins
Infections/sepsis
Lifestyle factors, e.g. stress, sleep deprivation, sleep apnea
Genetic factors
Trauma
Electrolyte imbalances
High blood pressure
Surgery
IV fluids
Aging



GlycoCheck

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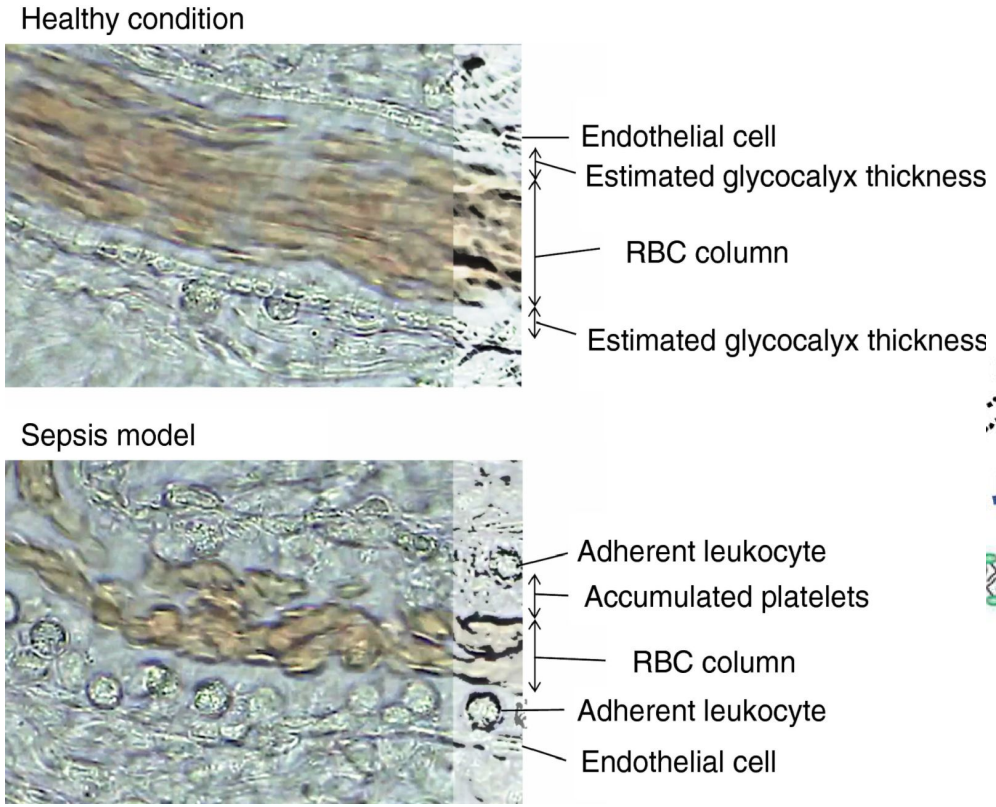
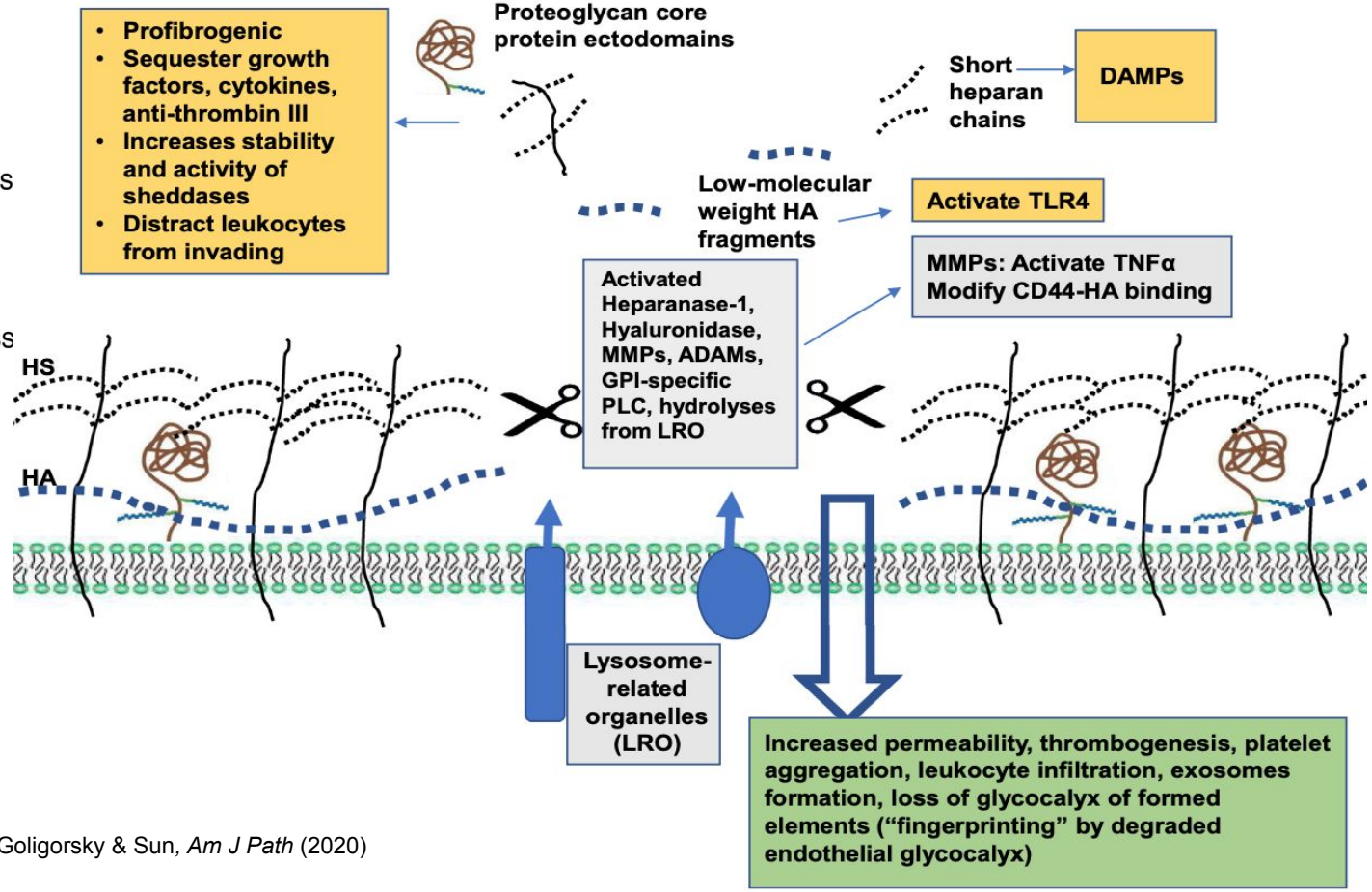
Endothelial glycocalyx in sepsis

- Severe generalized infection >>> systemic multiorgan sequelae
- Key molecular cause of gram (-) is outer membrane glycan LPS >>> inflammasome induction
- Increases sheddases and glycocalyx destruction.



Iba & Levy, *J Thromb Haamost* (2019)



Goligorsky & Sun, *Am J Path* (2020)

EGX in COVID-19

Early evidence:

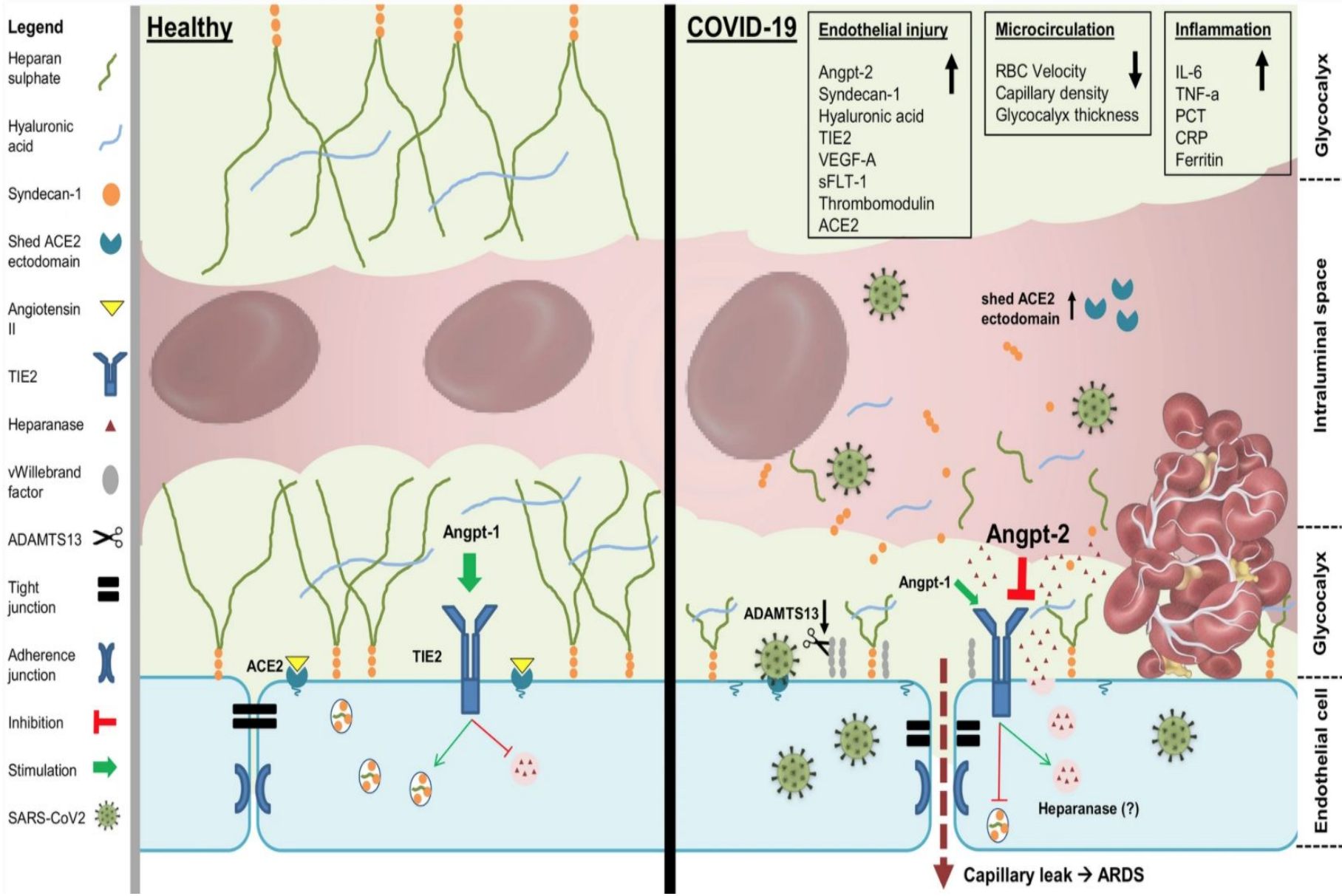
Reduced total and perfused vascular density in sublingual microvessels in ventilated patients

MYSTIC: Microvascular dysfunction in COVID-19

Mod-sev or critical

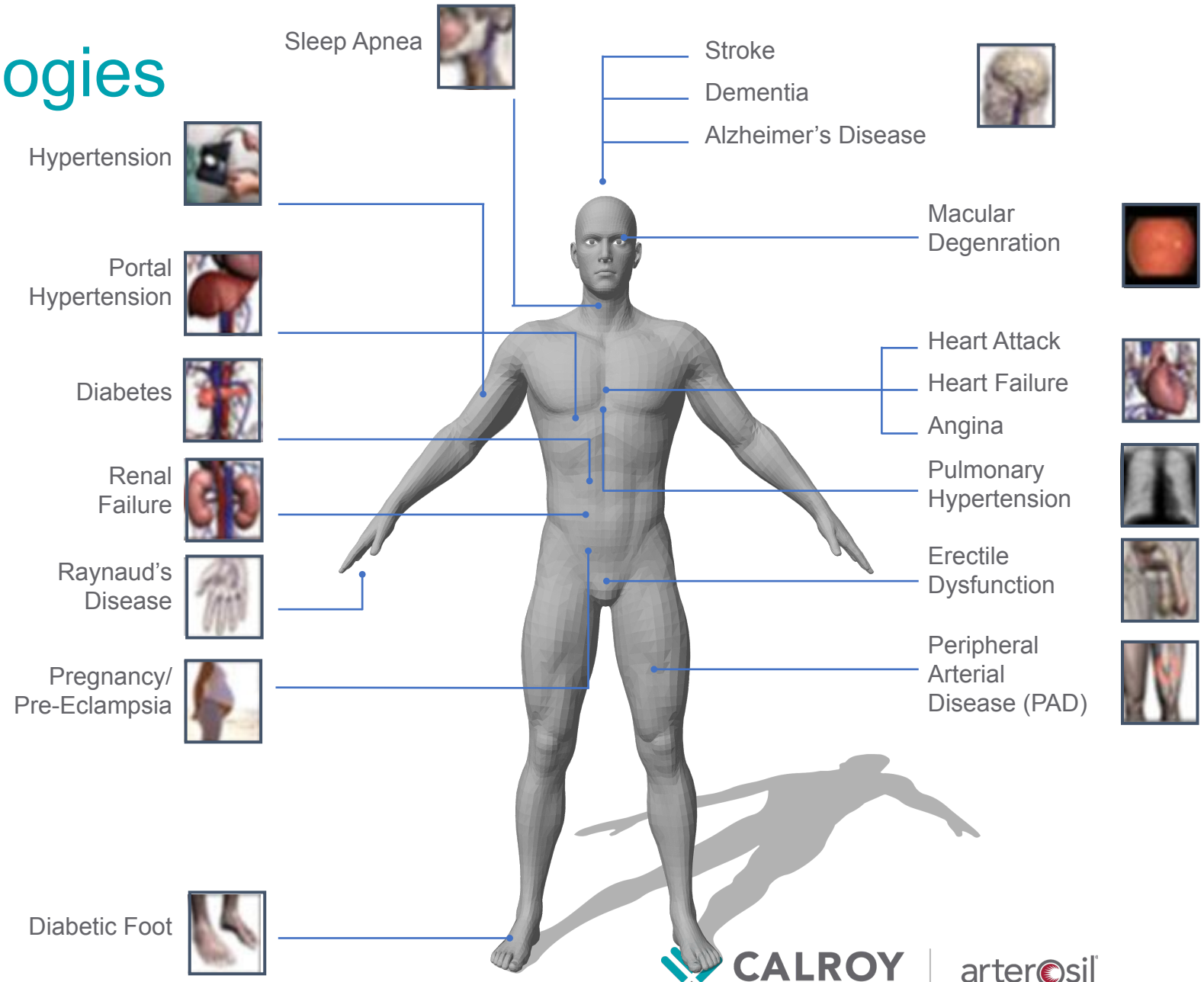
Intravital microscopy + circulating markers

Replicated early evidence + direct eGCX damage



Associated pathologies

- Cardiovascular disease
- Stroke
- Atherosclerosis
- Peripheral artery disease
- Renal disease
- Hypertension
- Diabetes, diabetic neuropathy
- Erectile dysfunction
- Other circulatory issues



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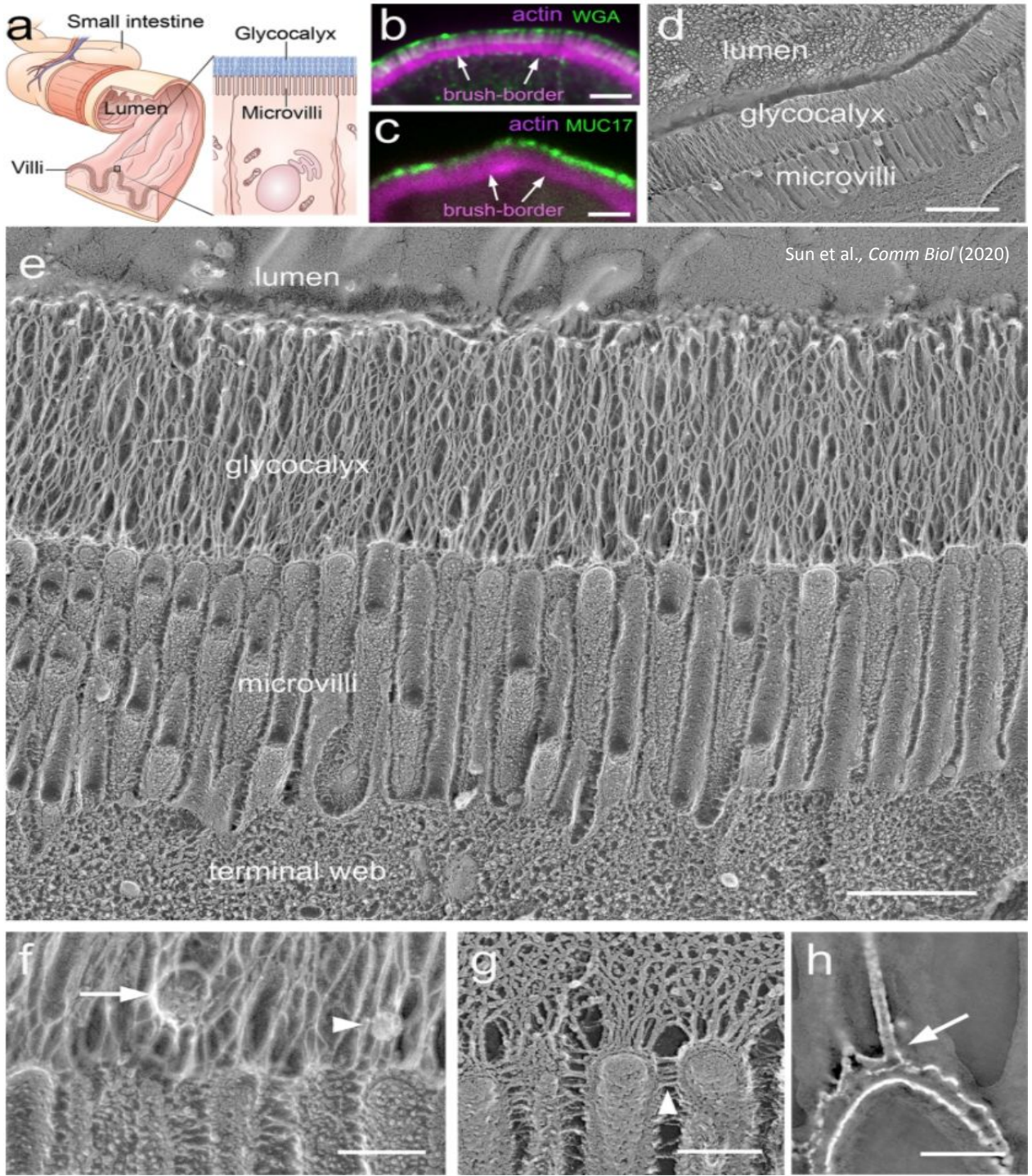
Glycocalyx functions in the gut

- Secreted and transmembrane glycoprotein layers over entire tract; protects mucosa from pathogens, mechanical stress
- In small intestine, covers entire epithelia cell surface; overlaying mucus layer can be thin and discontinuous
- Highly diverse glycoproteins, glycolipids act as receptors for bacterial adhesion, including normal flora to limit pathogen colonization
- Mucosal lubrication and hydrophobicity; prevents auto-digestion and ulceration; participates in cell signaling; selective diffusion barrier to exclude deleterious microbes

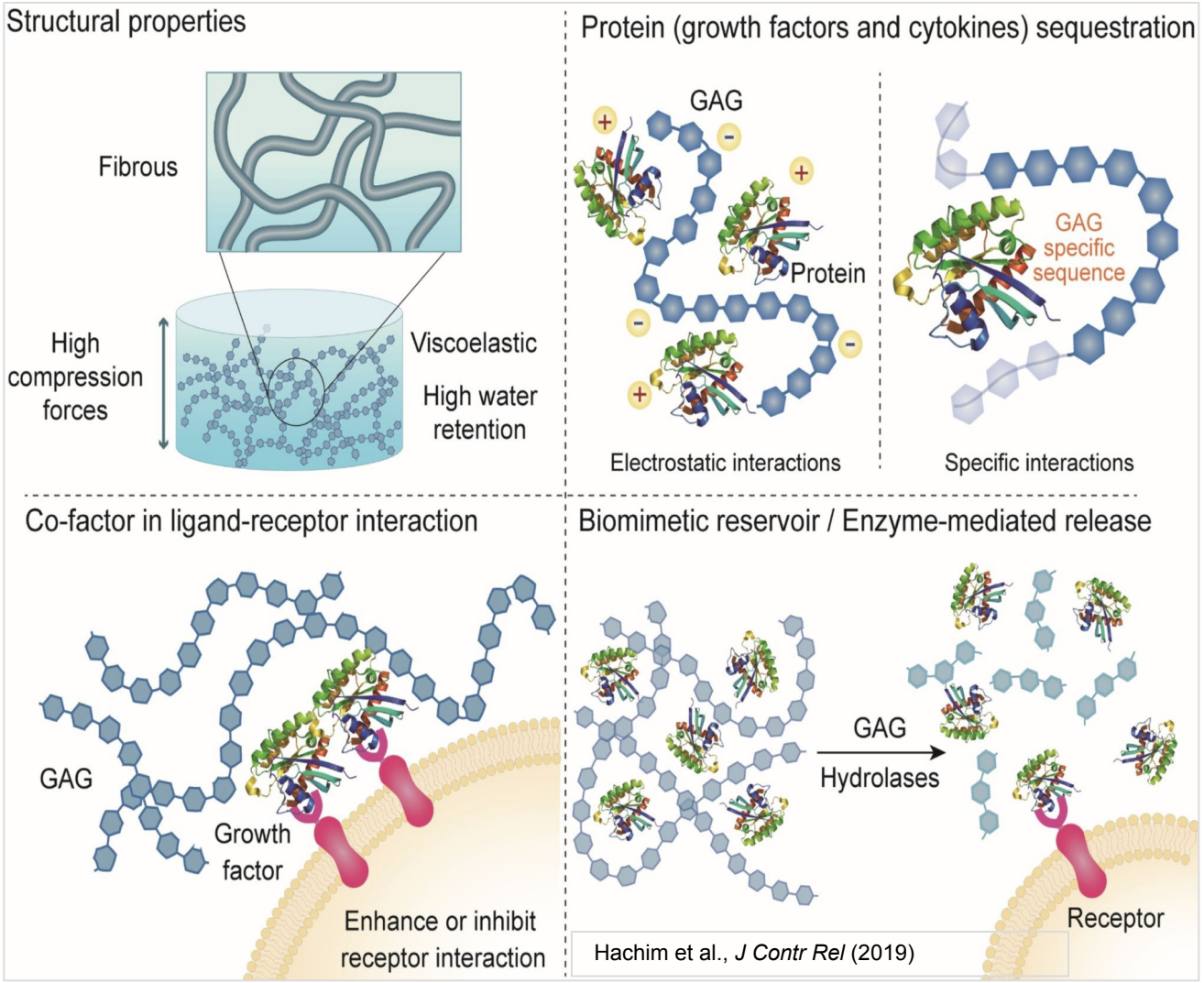
IN THE CLINIC

GCX impairment implicated in several GI diseases, e.g. IBD, cancer



Intestinal Glyco-therapeutics

- N-acetyl glucosamine (GlcNAc) tested in a phase 2 trial with IBD
- Oral GlcNAc was reported to promote mucus production in intestinal tissues of children with severe treatment-resistant IBD
  - 8 of 12 children given GlcNAc achieved clinical remission
- GlcNAc is an immunomodulatory agent and promoter of epithelial barrier integrity



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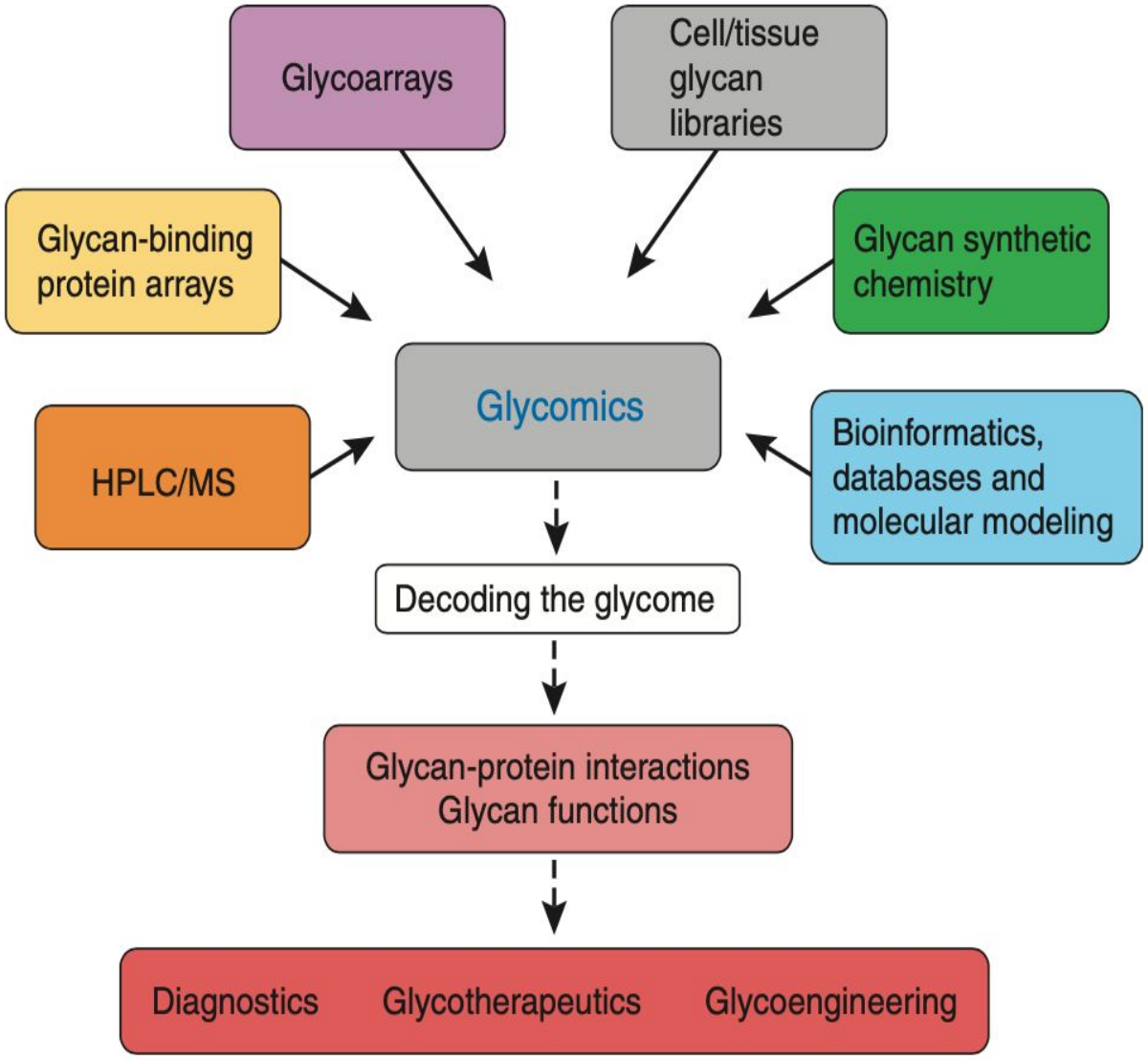
Glycomics: Approaches and Technologies



Turnbull & Filed, *Nat Chem Bio* (2007)

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Diagnostics: Clinical glycan profiling

Tegtmeyer et al., *NEJM* (2014)

<u>CDG Screening</u>:

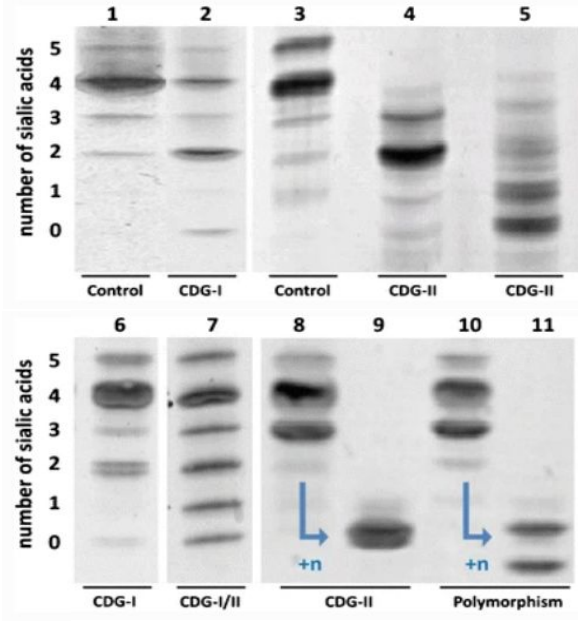
Carbohydrate-deficient transferrin (IEF)
Apo CIII
Mass spec (MS)

<u>CDG Advanced</u>:

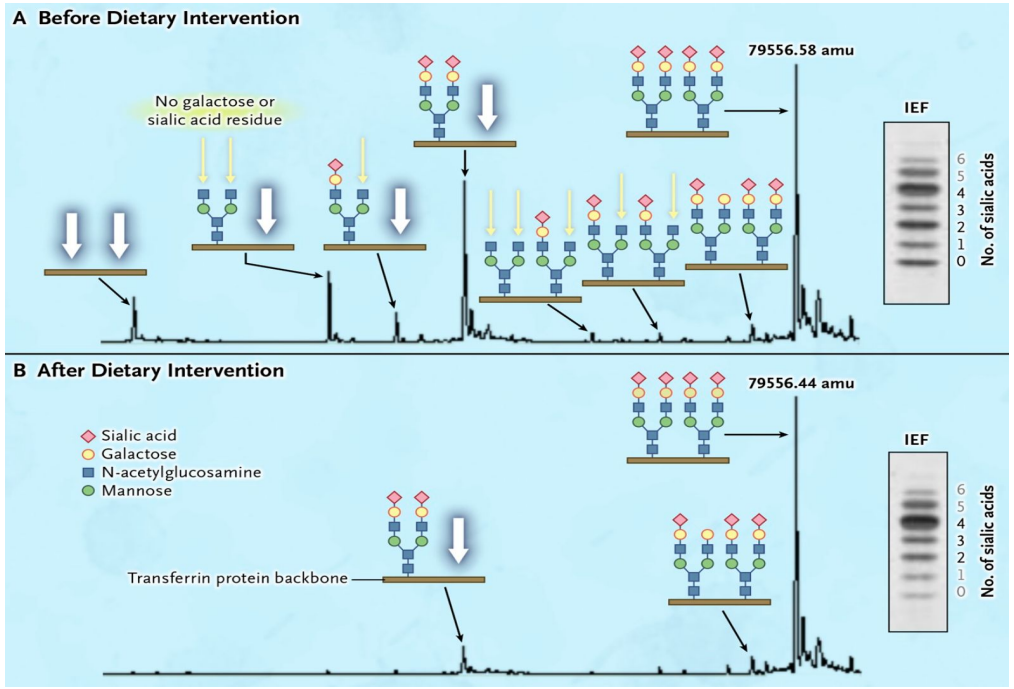
WES/WGS >>> subtle phenotypes, discovery of novel genetic causes, non-global functions

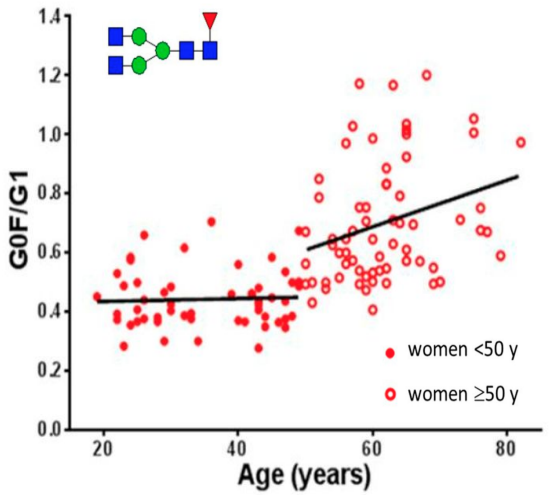
<u>Direct-to-consumer</u>:

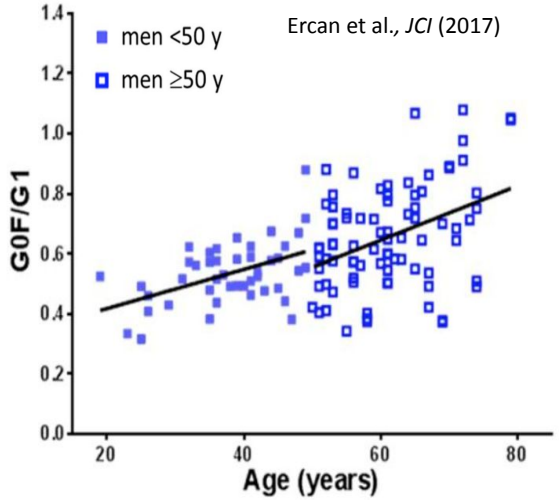
Biological age



Van Scherpenzeel et al., *Glycocon J* (2014)







Ercan et al., *JCI* (2017)

Microarrays, global glycan profiling

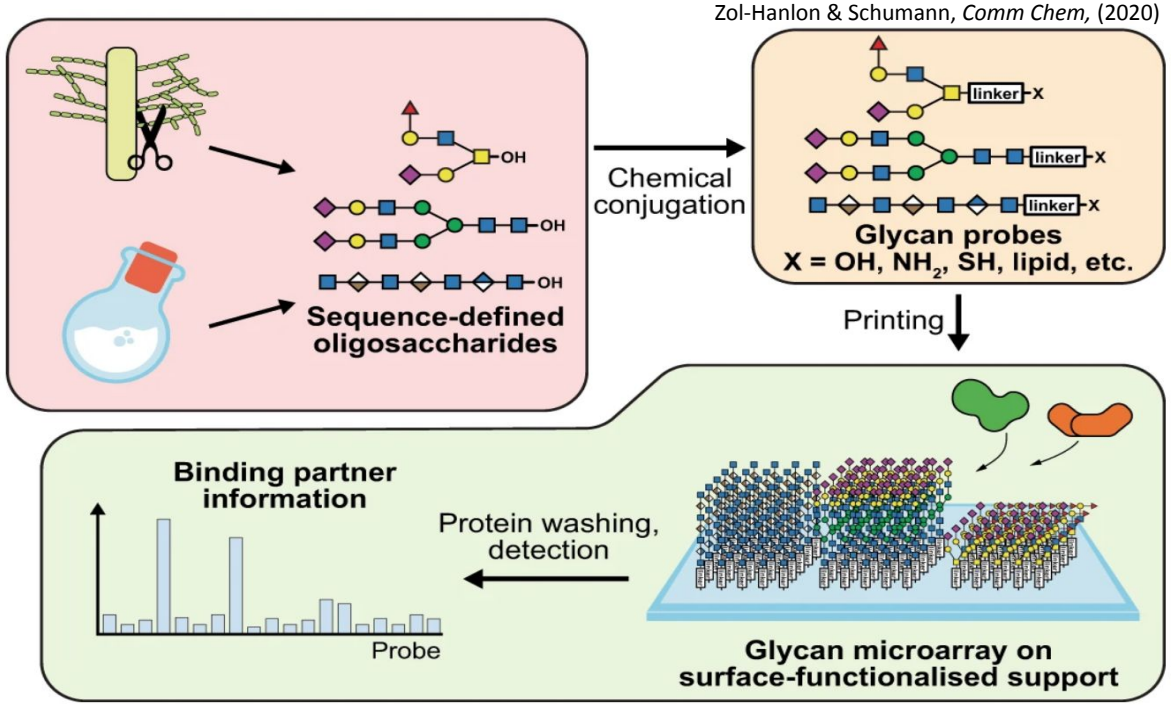
Glycans, lectins, mucins:

State-of-the-art, key resource for many years
Limited by number of pre-specified glycans (~100s) vs. 1000s possible *in vivo*

IN THE CLINIC

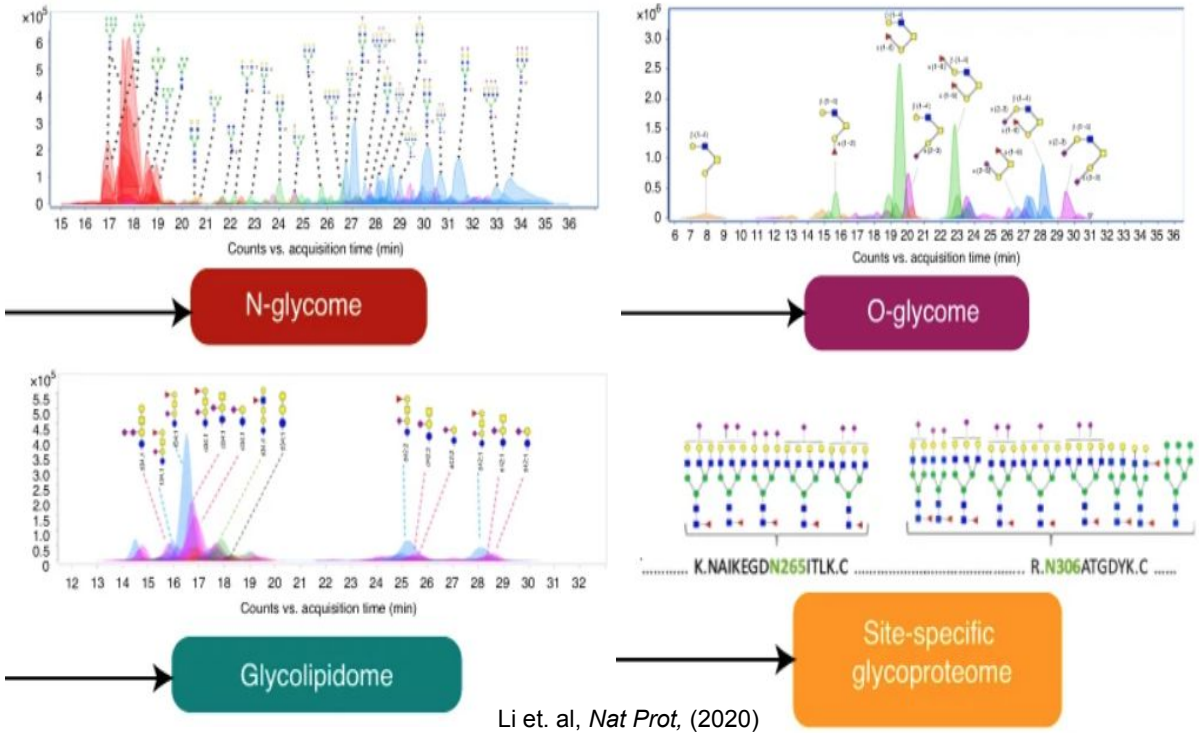
Glyco Liver Profile

Zol-Hanlon & Schumann, *Comm Chem,* (2020)



Next generation:

Multiplex bead arrays – boost throughput
Cell-based arrays – near-native environment
Liquid arrays – densely conjugated, DNA-barcoded virions
Parallel shotgun glycomics + proteomics
Site-specific glycopeptide mapping



Li et. al, *Nat Prot,* (2020)

Diagnostics: Endothelial status and function

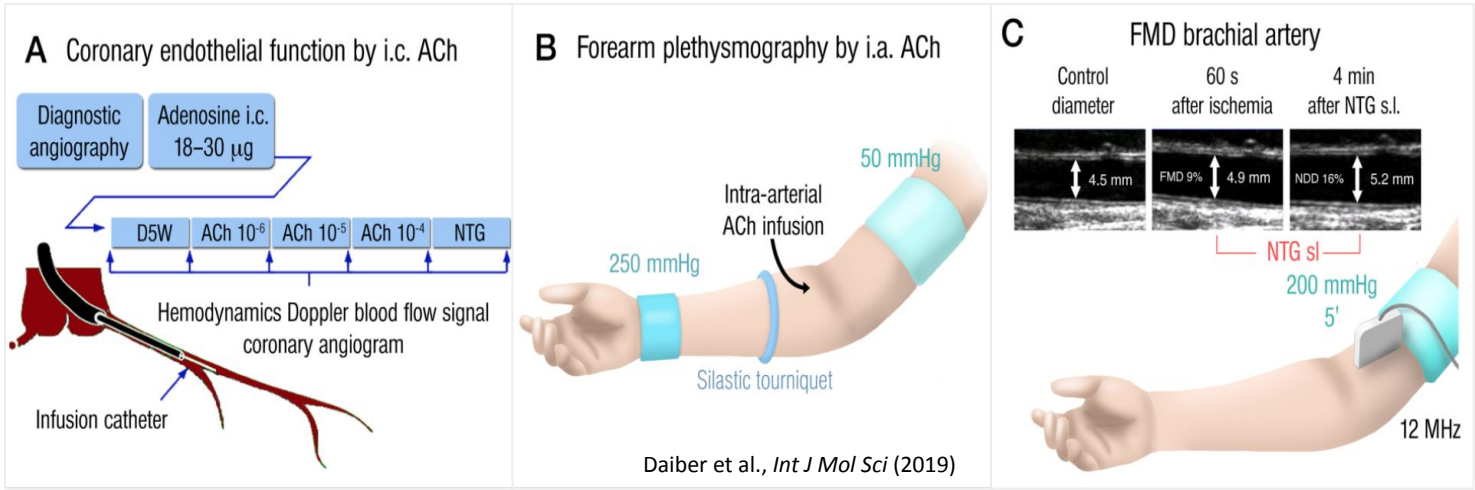
Previous methods are invasive and/or challenging to administer

EndoPat – reactive hyperemia in finger microvasculature

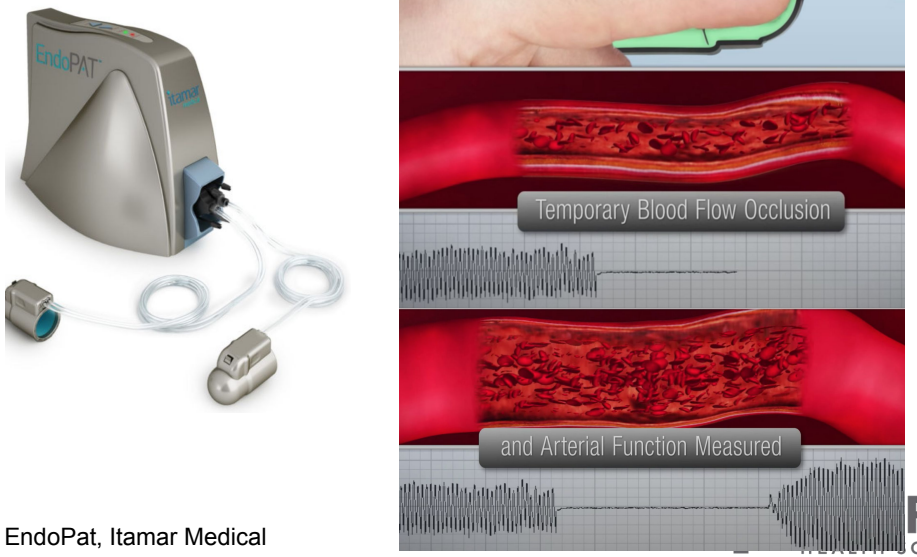
Easy to access and perform
Automated
Low interobserver and intra-observer variability
Correlation with invasive microvascular vascular function

Pulse wave velocity – volume wave form information via finger probe

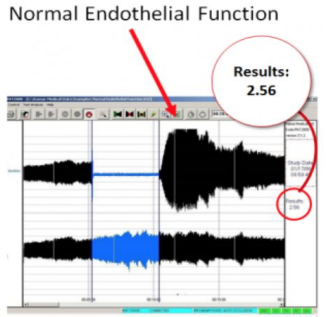
Arterial stiffness
Vascular tone

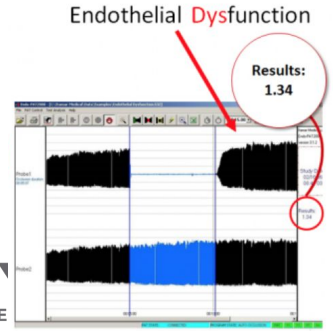


Daiber et al., *Int J Mol Sci* (2019)



EndoPat, Itamar Medical

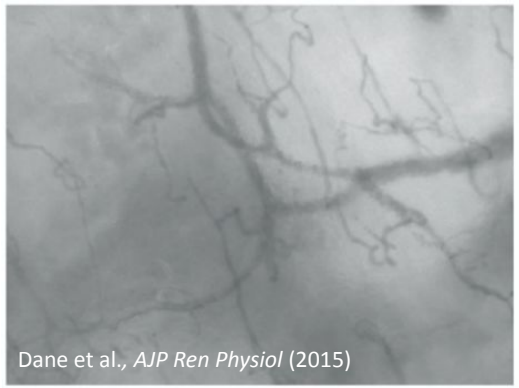


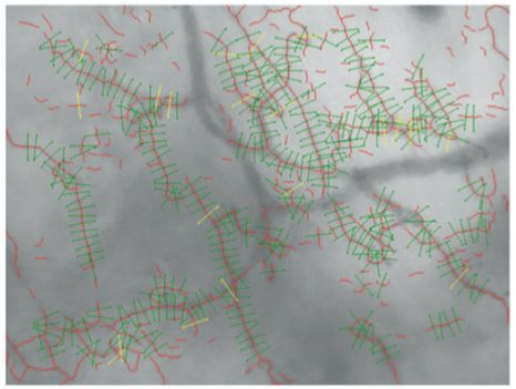


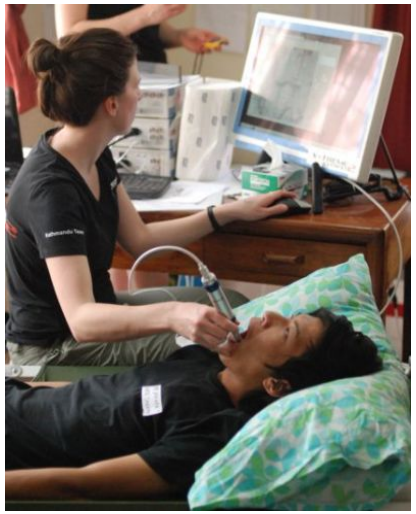
Sidestream dark field imaging allows real-time eGCX assessment

- Hand-held videomicroscope records sublingual mucosal microvasculature (5-25 μm)
- Software automatic calculation of measurements:
  - capillary density
  - capillary blood volume
  - blood flow
  - red cell velocity

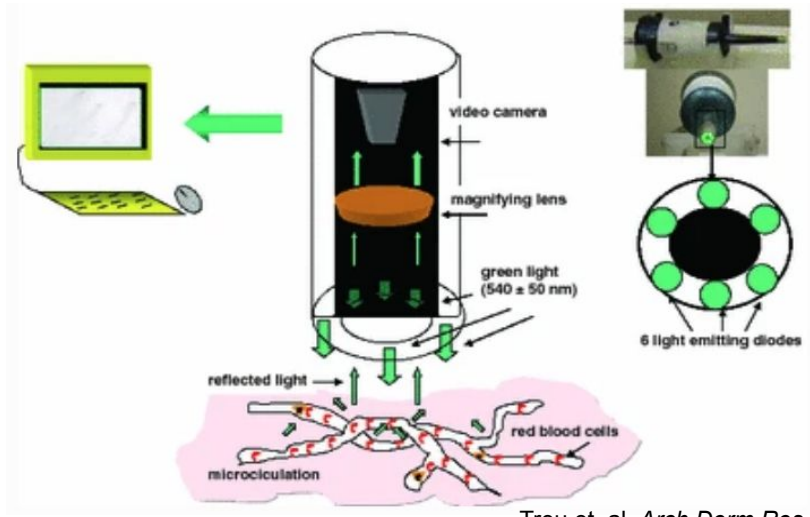
Perfused boundary region (PBR) =
inversely proportional to EGX thickness



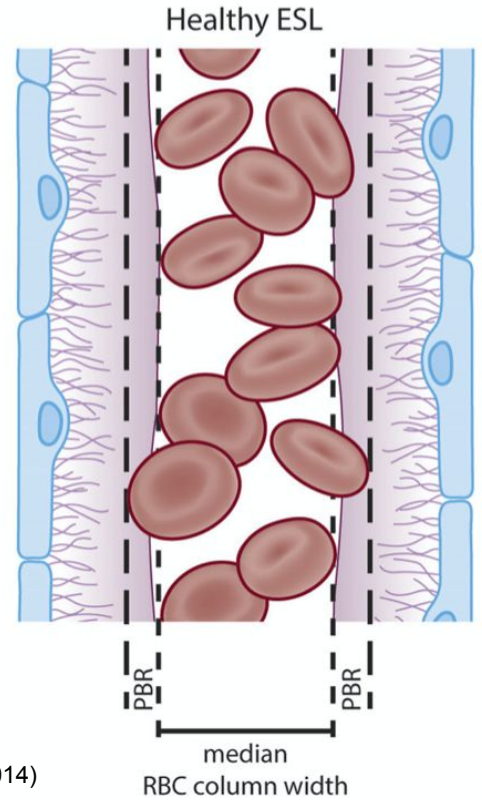


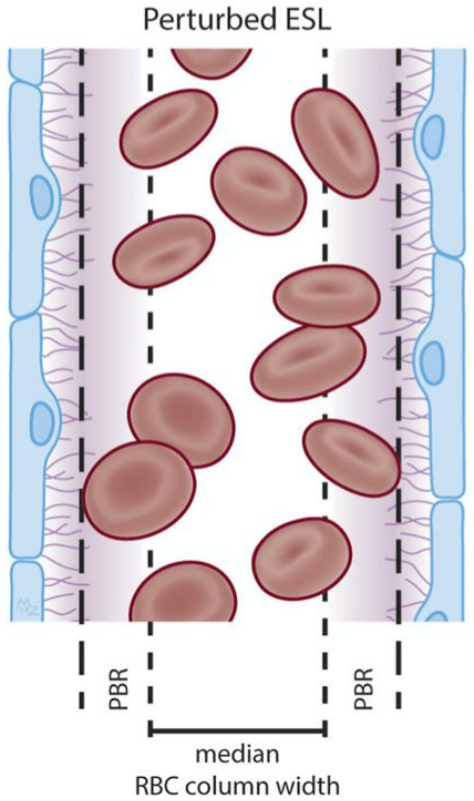


Pring, F., *BMC Blog* (2013)



Treu et. al, *Arch Derm Res* (2014)





Endothelial glycocalyx is a therapeutic target

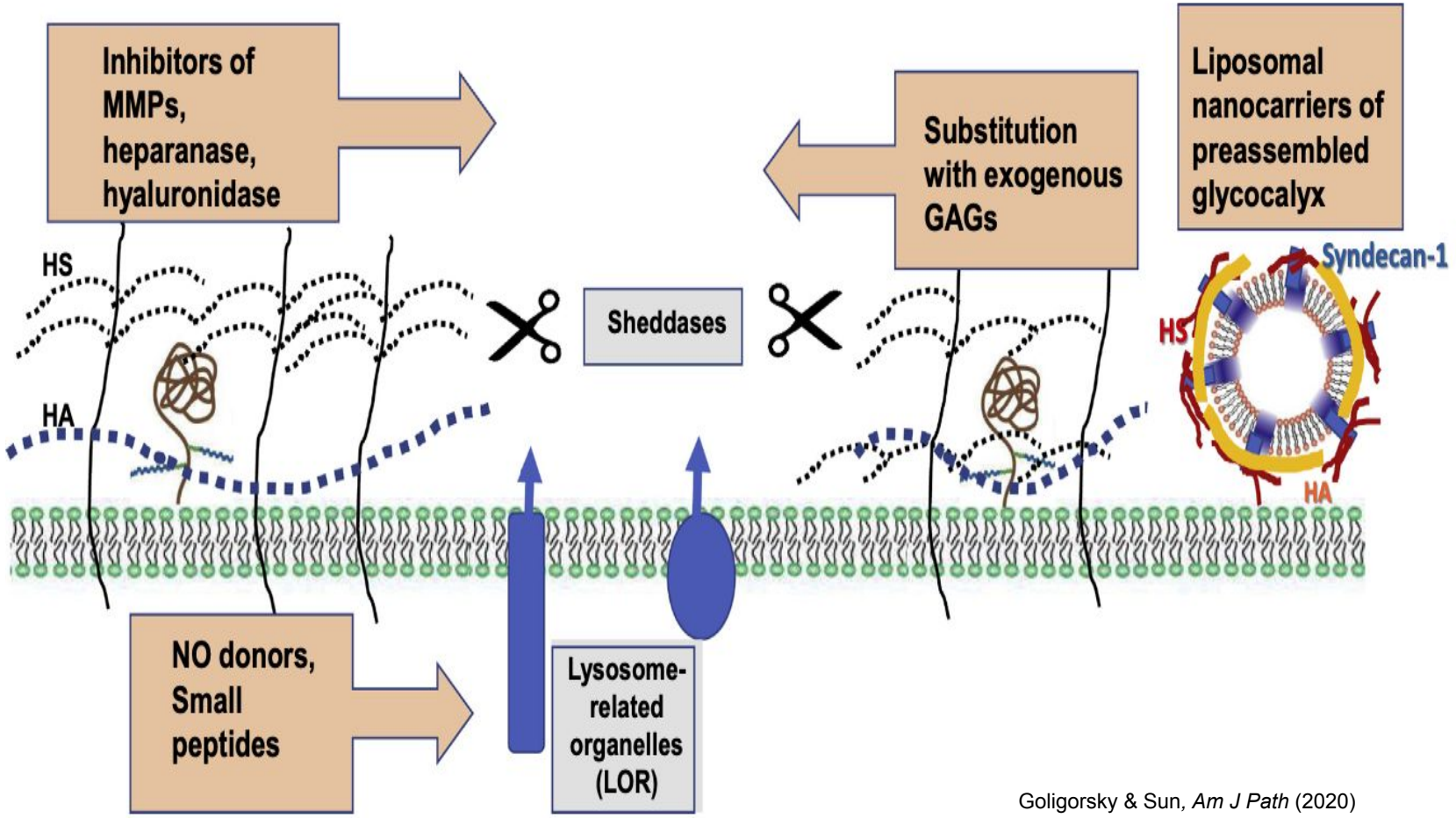
Restoration
requires days (5-7) to achieve functionality after an insult

Preservation
precipitous degradation, exigent requirement for restitution

acute I-R, sepsis, burns, surgical stress, aortic clamping / CV bypass, toxin-producing infection

gradually accruing imbalance between synthesis-degradation, leading to defects

DM, chronic CV diseases, CKD, tumors, chronic inflammatory processes, aging



Goligorsky & Sun, *Am J Path* (2020)

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EGX is a therapeutic target

<u>Existing compounds</u>:

albumin

FFP – preserves integrity and SYND1, source of albumin, protease inhibition, induces endothelial cell release of preformed SYND1

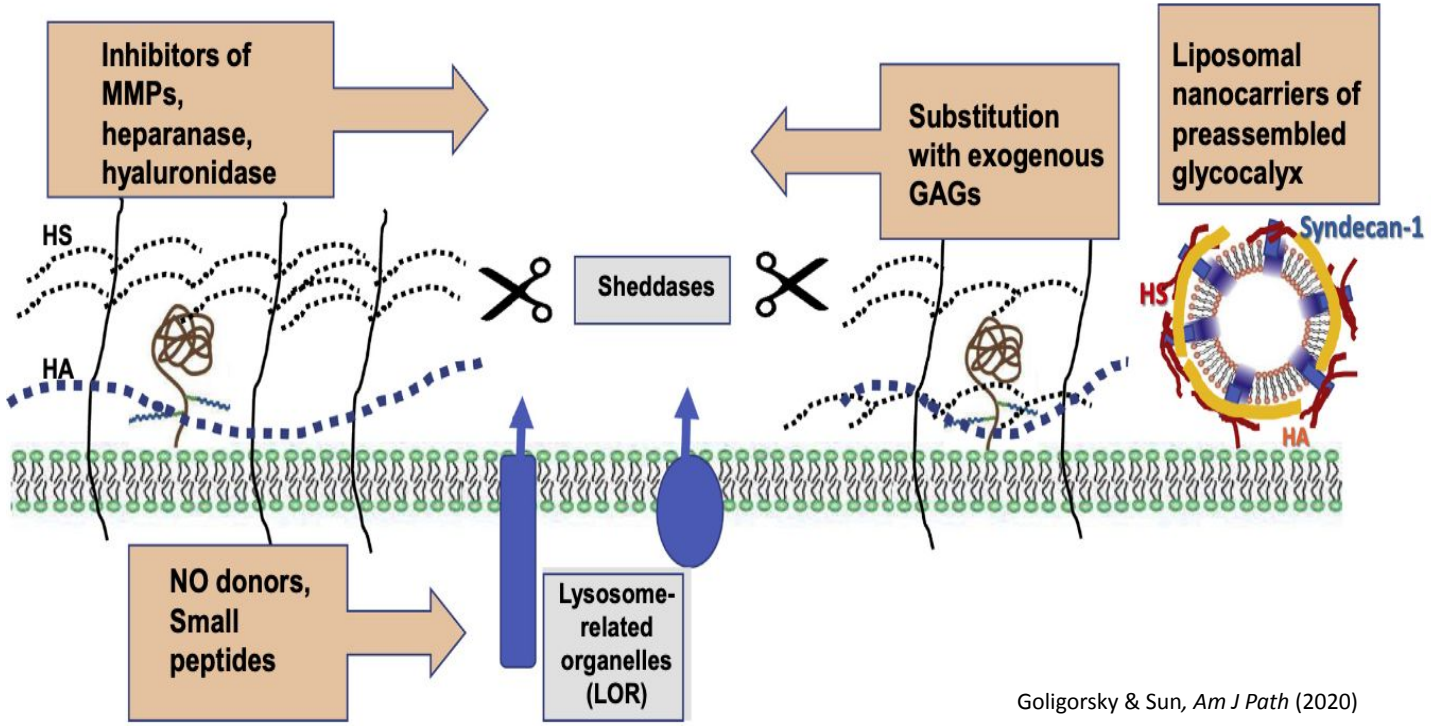
glucocorticoids – anti-inflammatory actions, incl cytokine-induced vascular barrier damage on vascular barrier

systemic high MW hyaluronic acid (HA)

HA infusion + chondroitin sulfate

doxycycline – MMP inhibition

NAC – opposes shedding in hyperglycemia



Goligorsky & Sun, *Am J Path* (2020)

<u>Novel compounds</u>:

heparin aggregates (Corline) – anti-thrombotic

selectin-targeting anti-adhesive coating (EC-SEAL) – dermatan sulfate backbone + selectin-binding peptides

Heparin-mimetics (Sulodexide) – HS + dermatan sulfate GAG, sheddase inhibitor

S1P/Albumin S1P –sheddase inhibitor

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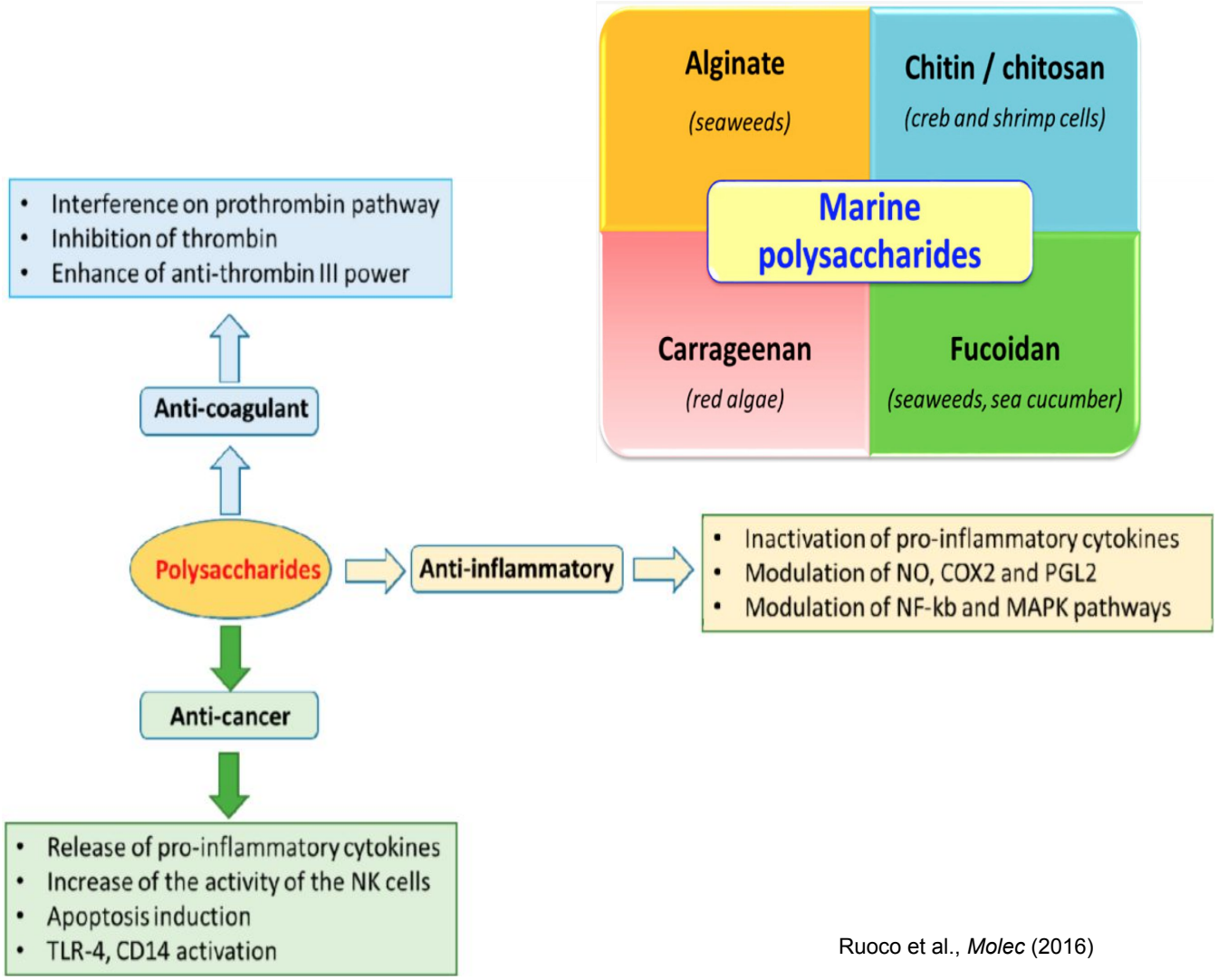
Glyco-therapeutics



Ruoco et al., *Molec* (2016)

Rhamnan sulfate, a sulfated polysaccharide from green seaweed

Rapidly absorbed and incorporated into the endothelial glycocalyx

Improves endothelial function

Anticoagulant

Antithrombotic

Antiviral effects

Modulates intestinal immune responses

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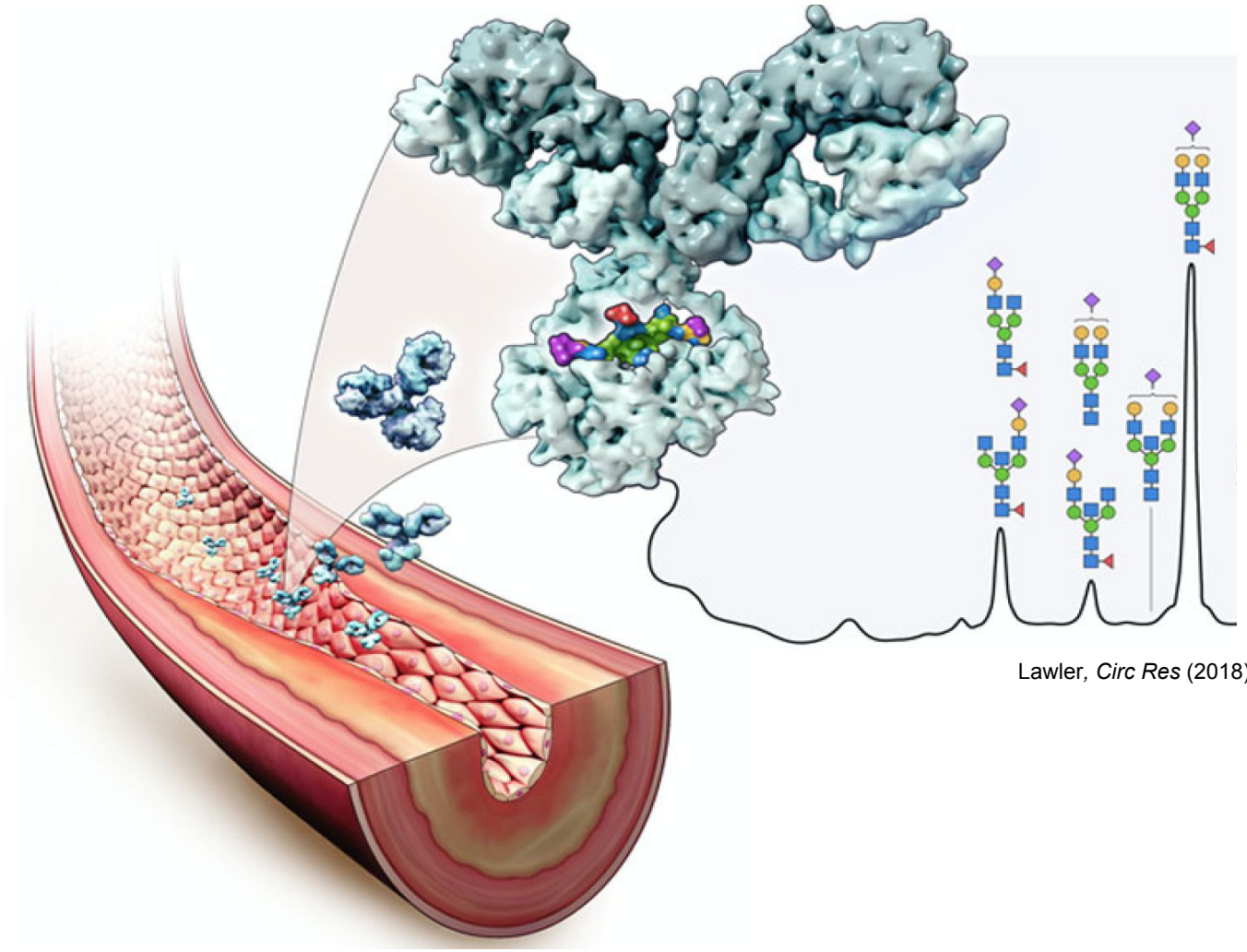
THANK YOU



Lawler, *Circ Res* (2018)

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