
CELL SELF-RECOGNITION
BY
GLYCONECTINS

During biological evolution two essential steps were necessary.

The first was the emergence of cells via molecular self assemblies, and the second subsequent one was the development of multicellular assemblies.

Multicellular life is the most sophisticated form of changing patterns of matter and energy known so far.

The emergence of multicellularity has been tightly coupled to the ability of an organism to retain its own anatomical integrity and to distinguish self from non-self.

This process required the simultaneous development of cell adhesion and recognition properties.

Cell adhesion defines the form, physical state and consequently the functioning of all multicellular living systems.

Self is commonly consider in biology as syngeneic specific (genetically identical).

Non-self as either xenogeneic specific or allogeneic specific.

Xenogeneic specific: derived from an organism of a different species – genetically not identical.

Allogeneic specific: derived from separate individuals of the same species - genetically not identical.

Intermolecular binding forces between cell adhesion molecules (CAMs) are intrinsic properties of such cohesive structures.

Differences in the degree of binding strengths between diverse types of CAMs at the given environmental thermodynamic conditions determine selectivity of their associations.

Therefore, distinguishing self from non-self must operate via alogeneic and/or xenogeneic differences in types and/or spatial and temporal expression of cell recognition molecules and CAMs.

Information technologies are using principles of biological self/non-self discrimination in order to ensure the security of computer systems by fast detection of unauthorized access, assuring the integrity of data and preventing the spread of computer viruses.

These protection problems are instances of the more general problem of distinguishing self (legitimate users, corrupted data, etc.) from other (unauthorized users, viruses, etc.).

A change-detection algorithms based on the way that natural immune systems distinguish self from other were developed.

The sensor molecules guiding such recognition and adhesion should be present at the outermost cell surface.

What is the nature of the molecules operating in self/non-self discrimination?

Such molecules are biological polymers: proteins, glycans, lipids and their naturally covalently linked combinations glycoproteins and glycolipids including few organic monomeric molecules.

To understand the molecular basis of primordial self-recognition and non-self discrimination we focused our attention on the role of proteoglycan-like glyconectins in Porifera xenogeneic cell interactions, as the evolutionary most compatible model system for ancestors of Metazoans.

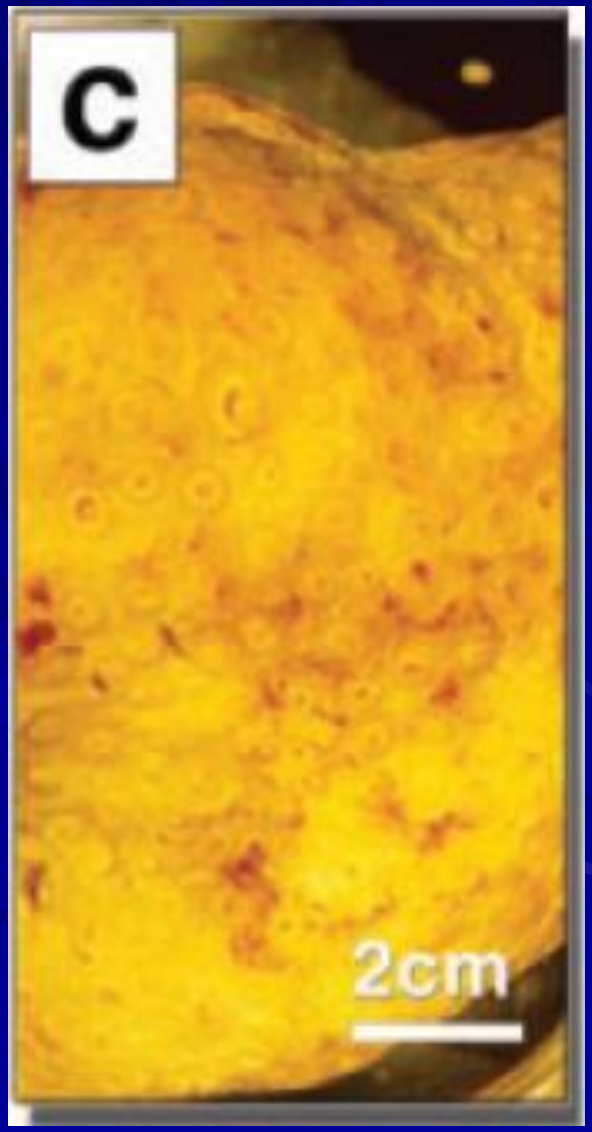
The molecular mechanism of self/non-self discrimination and adhesion in sponges should be most similar to the mechanism that operated during the emergence of multicellularity.

Living Marine Sponges

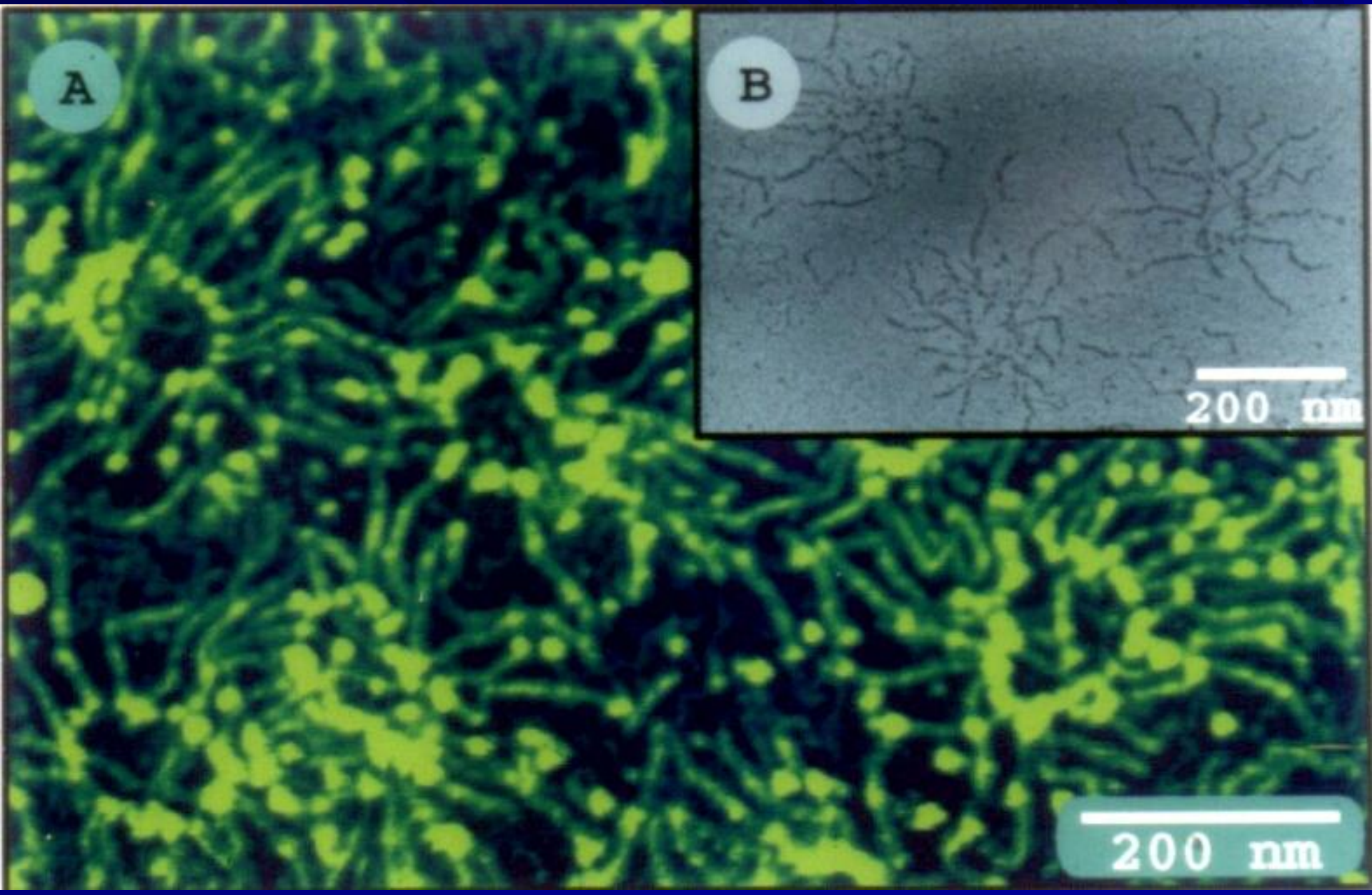
Clathria prolifera

Halichondria panicea

Cliona celata



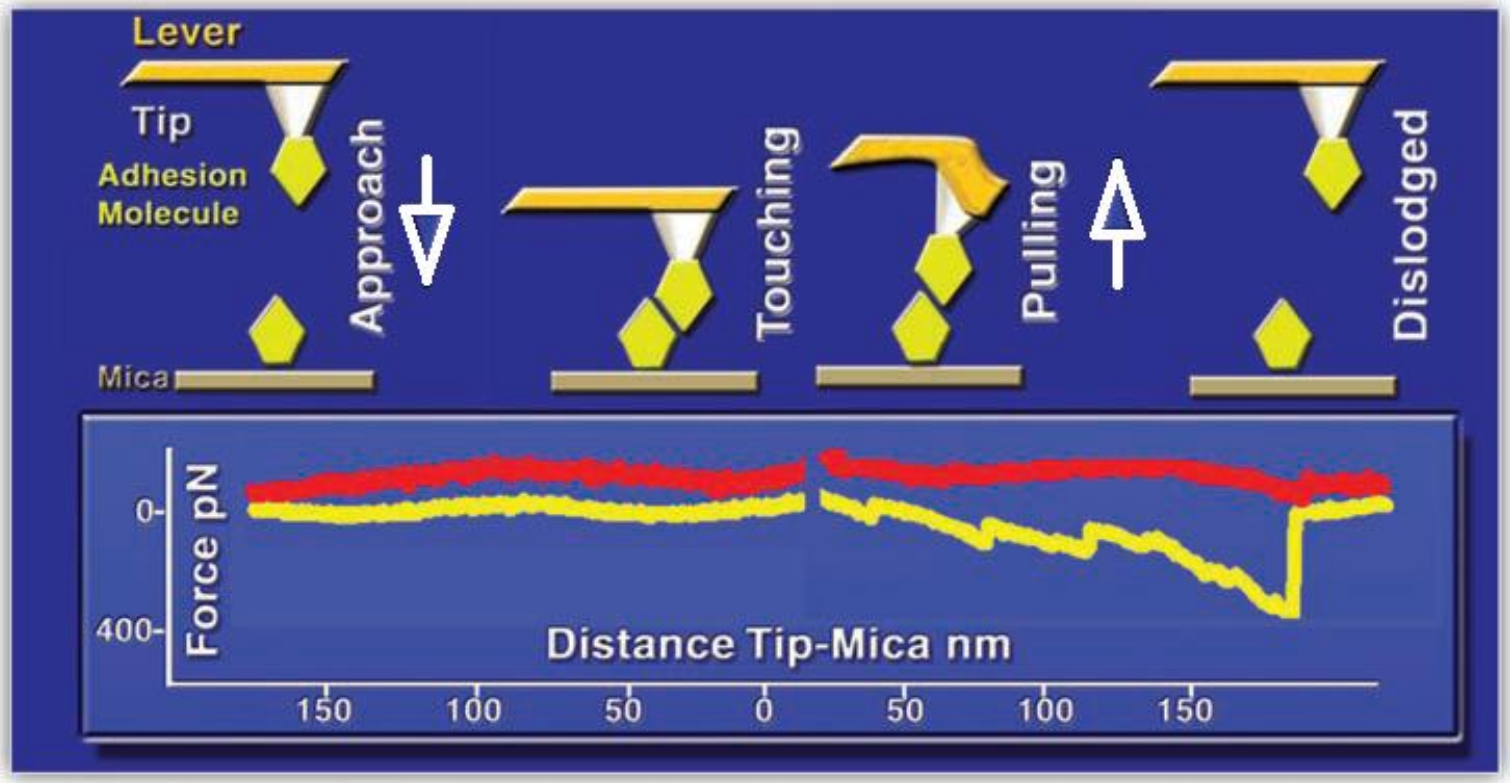
Our earlier investigations provided direct evidence that a novel class of primordial proteoglycans, named by us GLYCONECTINS (GNs), can mediate cell adhesion via a new alternative molecular mechanism of polyvalent glycan-glycan binding.

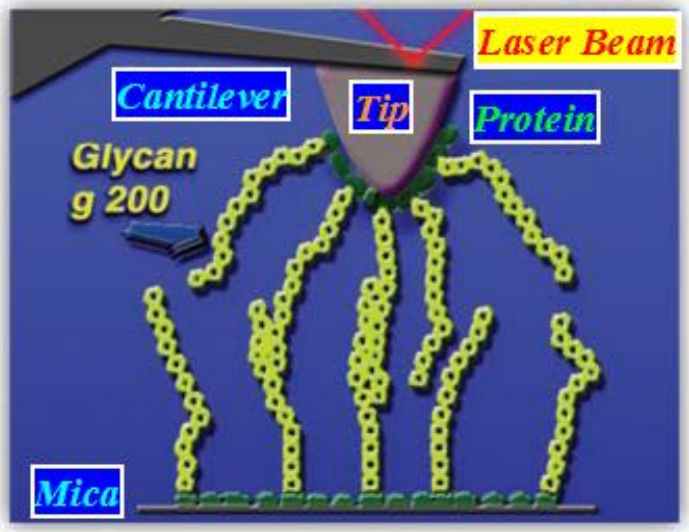

A
B
200 nm
200 nm

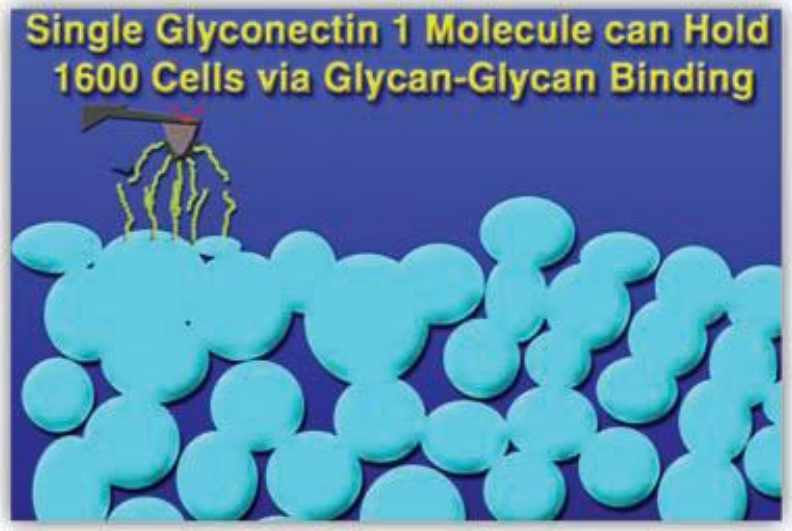
Porifera Glyconectins

Atomic force microscopy measurements demonstrated that the binding strength between a single pair of Porifera cell surface glyconectin 1 (GN1) glycoconjugates from *C. prolifera* can hold the weight of 1,600 cells, proving their adhesion functions.

Ca^{2+}-dependent self-interactions between glyconectin 1 molecules provide the major driving force for cell adhesion.


Lever
Tip
Adhesion Molecule
Approach
Touching
Pulling
Dislodged
Mica
Force pN
0
400
Distance Tip-Mica nm
150
100
50
0
50
100
150


Laser Beam
Cantilever
Tip
Protein
Glycan
g 200
Mica


Single Glyconectin 1 Molecule can Hold
1600 Cells via Glycan-Glycan Binding

Porifera Glyconectins

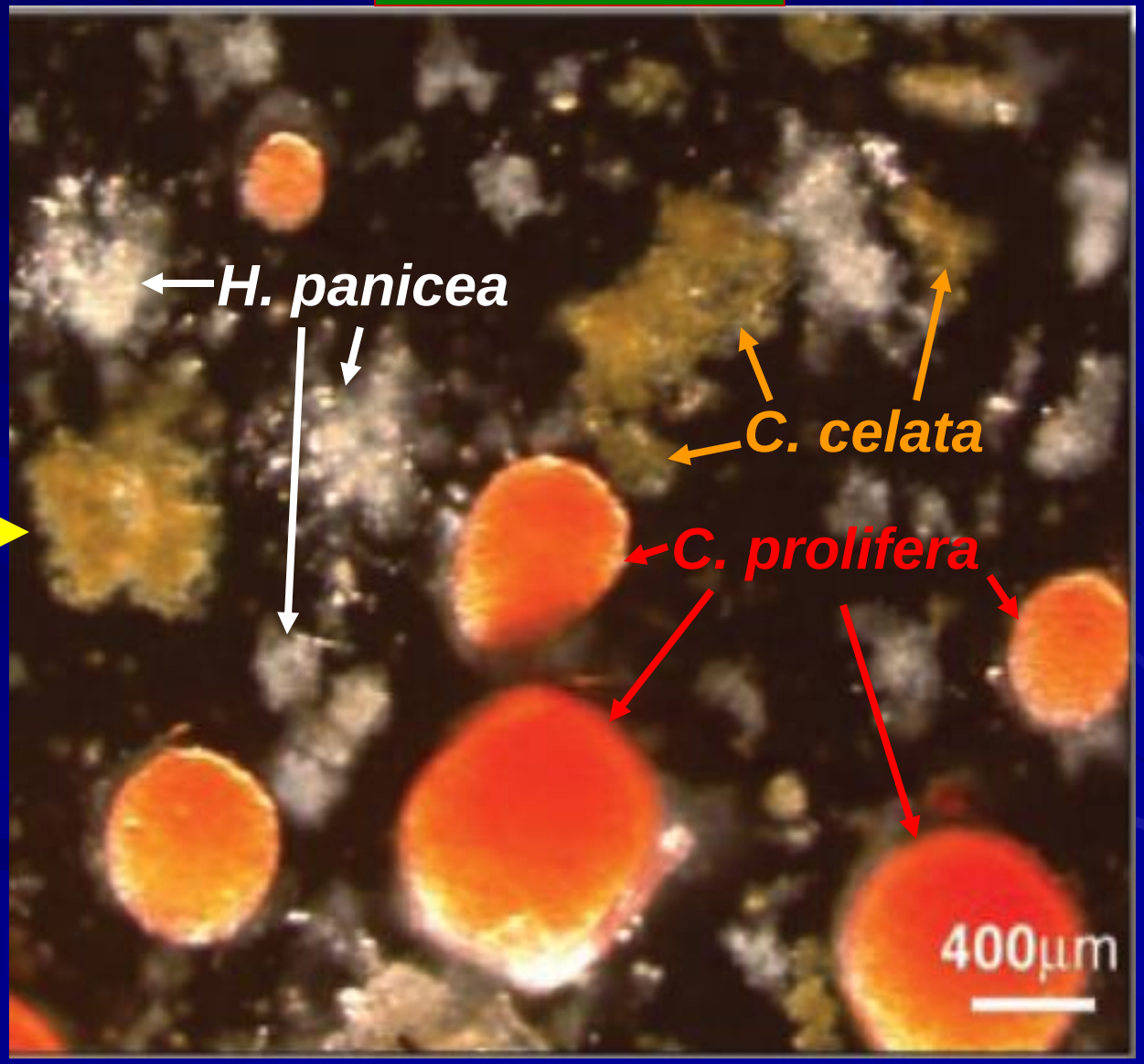
Using a quantitative functional approach with color-coded beads, cells, and blotting, we have shown that highly specific cell surface glyconectin to glyconectin interactions indeed mediate cell recognition and adhesion in the three Porifera species.

Glycoconjugates are cell adhesion and recognition molecules

Ca^{2+}-dependent glycoconjugate to glycoconjugate interactions mediate species-specific cell-cell recognition and adhesion

without Ca^{2+}

10 mM Ca^{2+}



10 mM Ca^{2+}

without Ca^{2+}

Simultaneous species specific

glyco nition

in ay

Porifera Glyconectins

Physicochemical and biochemical properties of glycans from three GNs, were established by extensive multidisciplinary approach:

- gel electrophoresis,
- ultracentrifugation,
- carbohydrate analyses,
- glycosaminoglycan-degrading enzyme treatment,
- NMR,
- mass spectrometry.

Physicochemical Properties of Sponge Glyconectins (GNs)

	GN1	GN2	GN3
$M_r \times 10^6$	19	10	8
$S_{20,w}$ (S)	58	42	46
Carbohydrate/ protein ratio (w/w)	63/37	21/79	36/64
Fucose (mol %)	28	7	31
Py(4,6)Gal (mol %)	4	16	0
GlcA (mol %)	11	3	6
SO_4^{2-} (mol/mol)	820	620	700

Monosaccharide Composition of GN Glycans

Monosac-charides	GN1 glycans (mol %)	GN2 glycans (mol %)	GN3 glycans (mol %)
Ara	0	0	7
Fuc	21	5	18
Man	6	13	13
Gal	30	37	16
GalNAc	2	0	8
GlcNAc	23	15	32
Py(4,6)Gal	8	26	0
GlcA	10	4	6

Porifera Glyconectins

The GN1, GN2, and GN3, showed species-specific carbohydrate compositions.

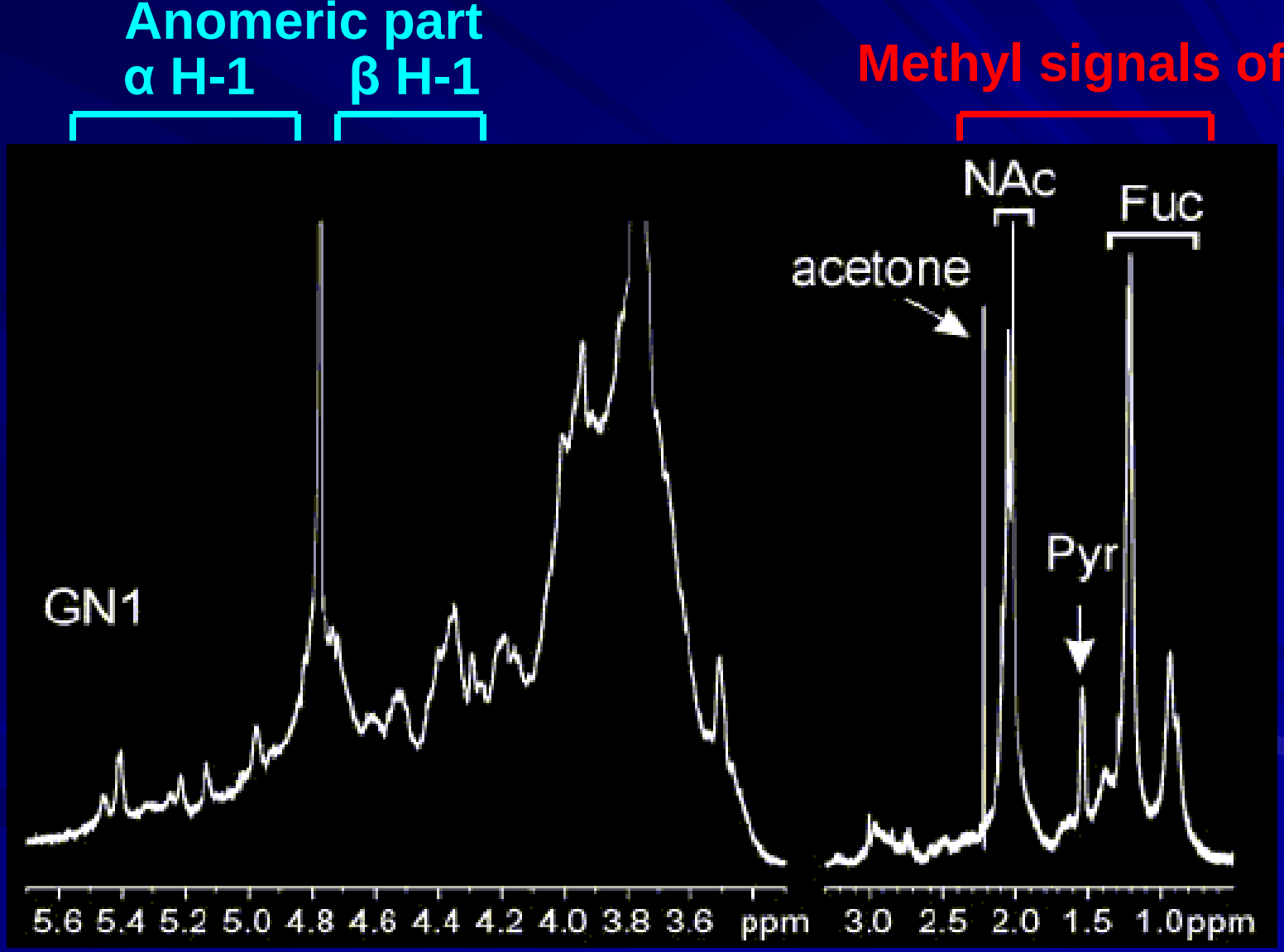
GN1 was characterized by a fucose content of one-third of the total monosaccharide content and minute amounts of Py(4,6)Gal.

GN2 had lower proportion of fucose and a molar ratio of Py(4,6)Gal three times higher than GN1.

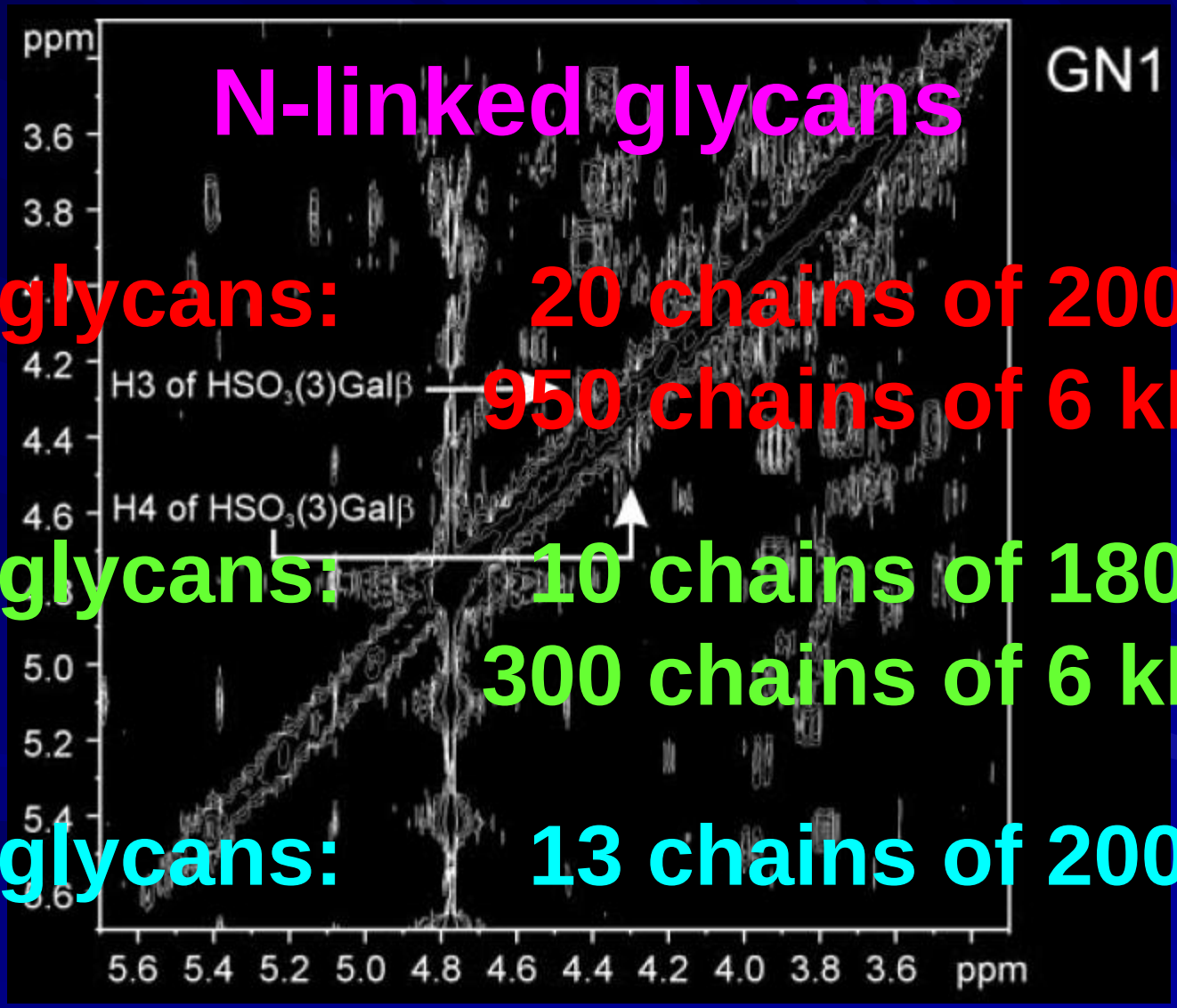
GN3 had high quantities of fucose but no Py(4,6)Gal at all and was the only GN to contain arabinose (7 mol %).

NMR Analyses of Glyconectin Glycans

- one-dimensional spectra -



NMR Analyses of Glyconectin Glycans
- two-dimensional COSY90 spectra -



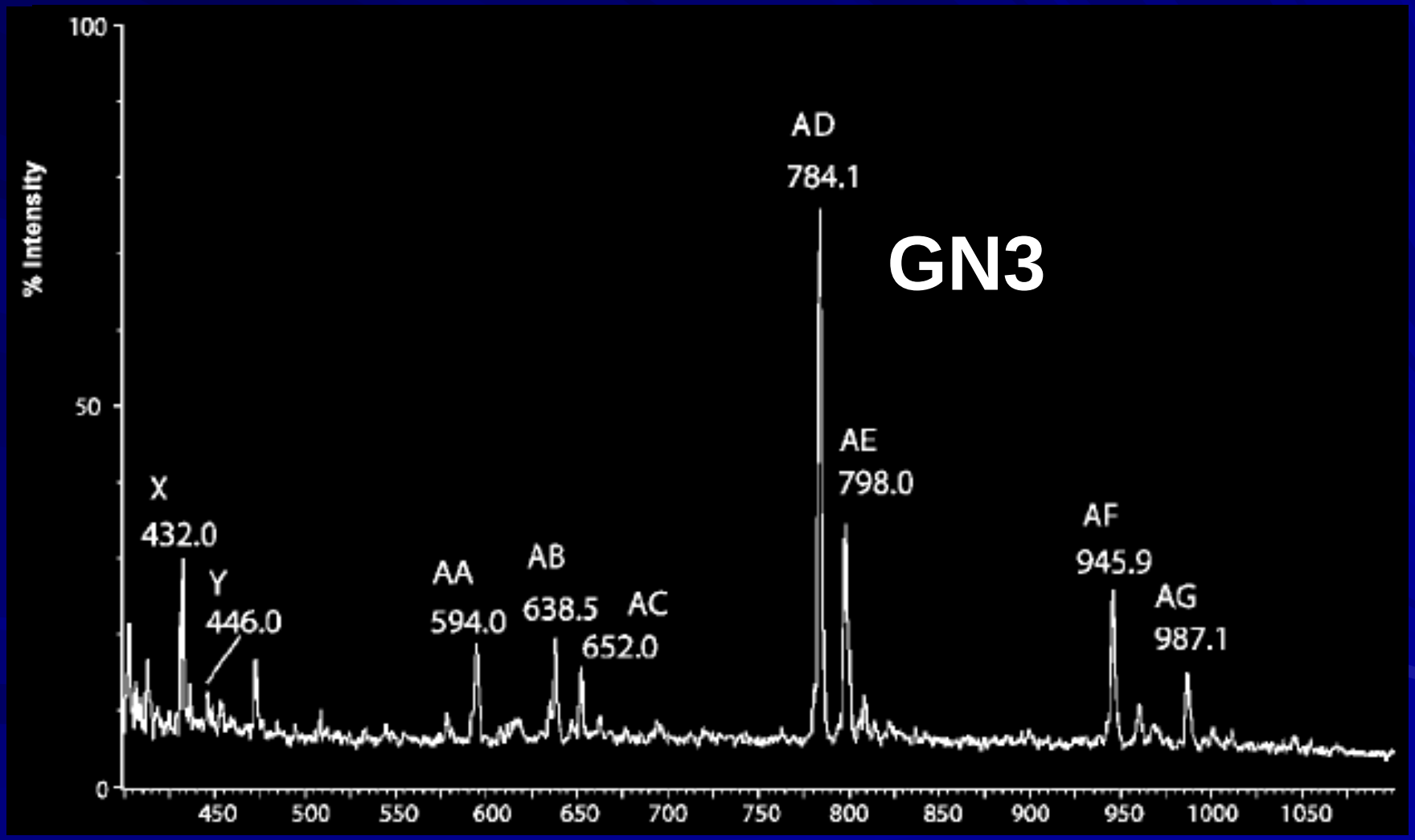
N-linked glycans

GN1 glycans: 20 chains of 200 kDa
950 chains of 6 kDa

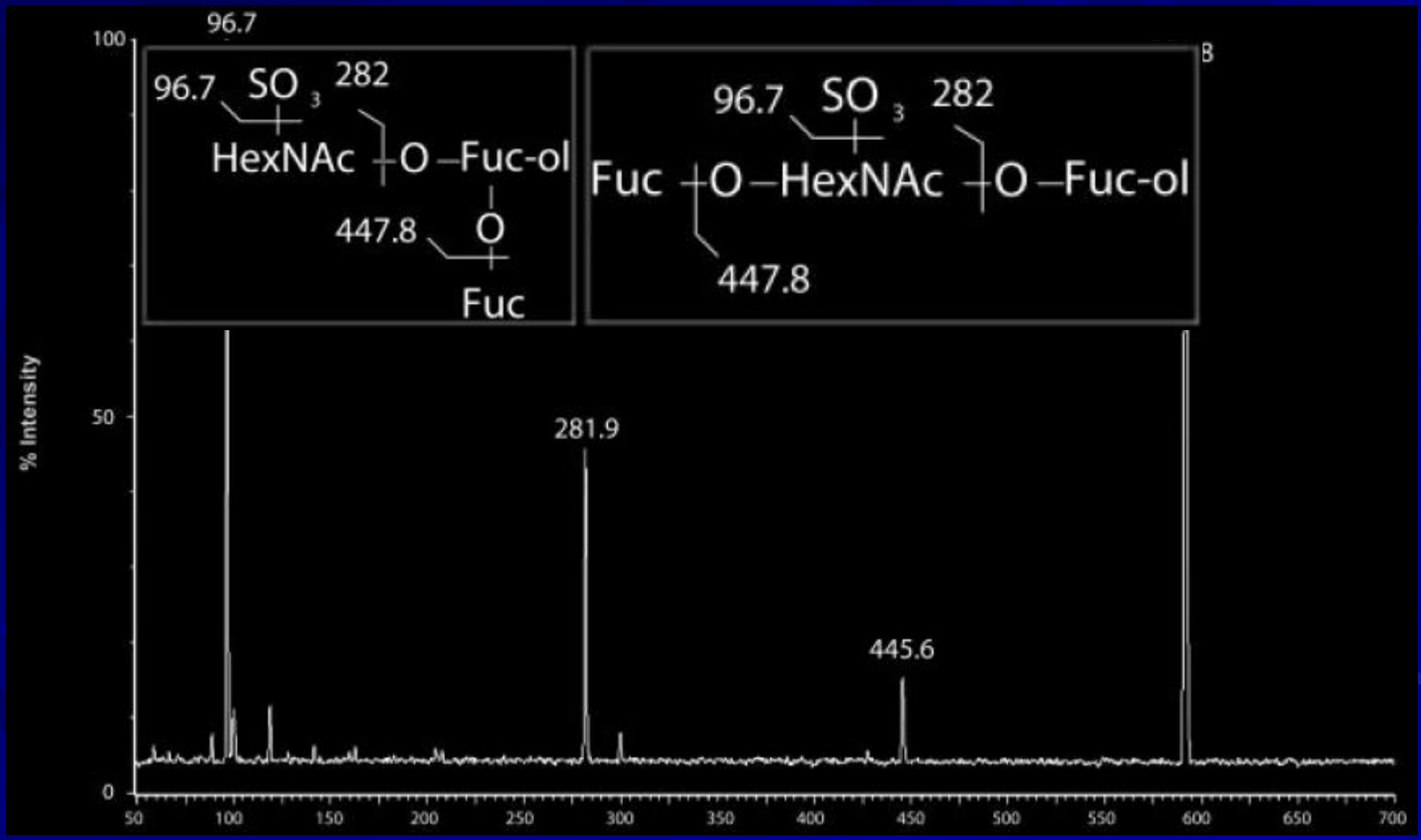
GN2 glycans: 10 chains of 180 kDa
300 chains of 6 kDa

GN3 glycans: 13 chains of 200 kDa

ES/MS profiling of ethanol-soluble oligosaccharides from purified glyconectins



ES/MS-MS fragmentation patterns in negative mode



Structural organization of Glyconectin 1 adhesion glycans

Porifera Glyconectins

The sequencing work presented here provides the first detailed comparative structural information on the glycan moieties of the glyconectins isolated from the *C. prolifera*, *H. panicea*, and *C. celata*.

Porifera Glyconectins

The sequential and selective chemical degradation of these three GN glycans, followed by mass spectrometric and NMR analyses of the fragments, resulted in chemical fingerprints revealing new types of species-specific structures:

- sulfated glycan sequences in GN1,
- branched pyruvilated sequences in GN2,
- linear sulfate structures in GN3.

Porifera Glyconectins

These data show also that GNs define a new family of proteoglycan-like molecules exhibiting species-specific structures with complex and repetitive acidic carbohydrate motives different from the classical proteoglycans and mucins.

Clearly the evolution from primordial Metazoans to complex organisms required the development of additional cell recognition and adhesion mechanisms mediated via lectin, immunoglobulin, integrin, and cadherin families of molecules.

Porifera Glyconectins

The specificity of carbohydrate-mediated homophilic GN interactions in Porifera approaches the binding selectivity of the evolutionarily advanced immunoglobulin (IG) superfamily.

However, the structural differences between these 2 systems imply conceptually distinct molecular mechanisms.

First, GNs are 100 times larger and extend 10 times farther from the cell surface than IG molecules.

Second, the GN1 specificity and tight binding of 10^9 M^{-1} reside in polyvalent glycan-glycan interactions of 1,000 sites, with a low affinity for the single site (10^3 M^{-1}), whereas IGs recognize antigens via higher affinity ranging from 10^4 to 10^9 M^{-1}, with low valency binding.

Porifera Glyconectins

Despite this evolving complexity of the components participating in cellular interactions, glyconectin-like structures have been preserved in mammalian systems, suggesting their versatility in recognition and adhesion.

Porifera Glyconectins

In conclusion, xenogeneic class of glyconectin glycans, as the most peripheral cell surface molecules of sponges (today's simplest living Metazoa), are proposed to be the primary cell adhesive molecules possessing self/non-self discrimination essential for the evolution of the multicellularity.

The GN-GN interactions, a veritable nano-velcro system, may thus provide a new model for molecular self-recognition.

Gradimir N. Misevic
Maurice Demarty
Camille Ripoll

Laboratoire des Processus Intégratifs Cellulaires, UMR 6037 CNRS, Faculté des Sciences et Techniques de Rouen, 76821 Mont St Aignan, Cedex, France

Gerard Strecker
Yann Guerardel
Marie-Christine Slomianny
Emmanuel Maes

Unité de Glycobiologie Structurale et Fonctionnelle, Université des Sciences et Technologies de Lille, UMR 8576 CNRS, 59655 Villeneuve D'Ascq, France

Yannis Karamanos

Laboratoire de Biochimie Moléculaire et Cellulaire, Université d'Artois, Faculté J. Perrin, rue J. Souvraz, SP18, 62307 Lens, France

Lazar T. Sumanovski

Department of Research, University Hospital of Basel, CH-4058 Basel, Switzerland