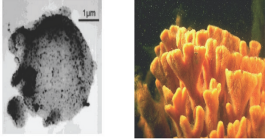


Carbohydrate-carbohydrate interactions

Marine sponges 1998-2009

- Primitive models for studying cell-cell recognition
- Species-specific aggregation of cells



*Microciona prolifera*

## Carbohydrate-Carbohydrate Interactions

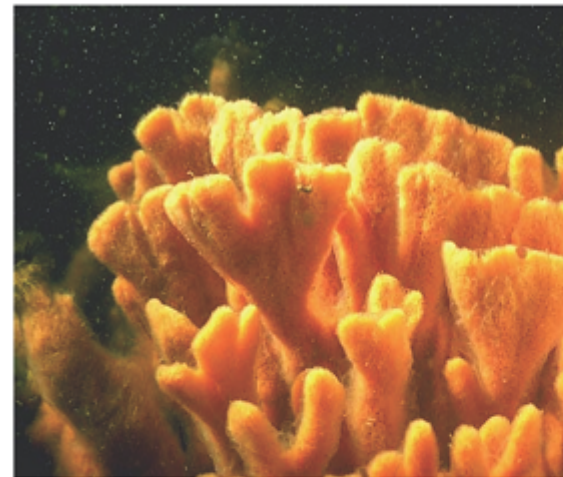
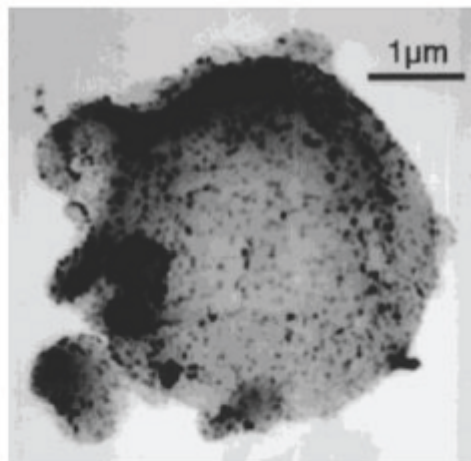
### Description

## Carbohydrate-carbohydrate interactions

### Marine sponges

199

- Primitive models for studying cell-cell recognition
- Species-specific aggregation of cells



*Microciona prolifera*

- When placed in artificial sea water free of  $\text{Ca}^{2+}$  ions, **complete dissociation** of the sponge tissue into a suspension of cells occurs.
- Formation of **cell-aggregates** by adding  $\text{Ca}^{2+}$  to the suspension; completely functional sponges can be the result.
- Cell-surface proteoglycans underlie the  $\text{Ca}^{2+}$ -dependent molecular mechanism of the self-recognition; coding **aggregation factors (AF)**.
- Sponge-species specificity of the aggregation process. **colour-specific sponge cell sorting**. Can be mimicked by **different coloured beads, coated with species-specific AFs**.

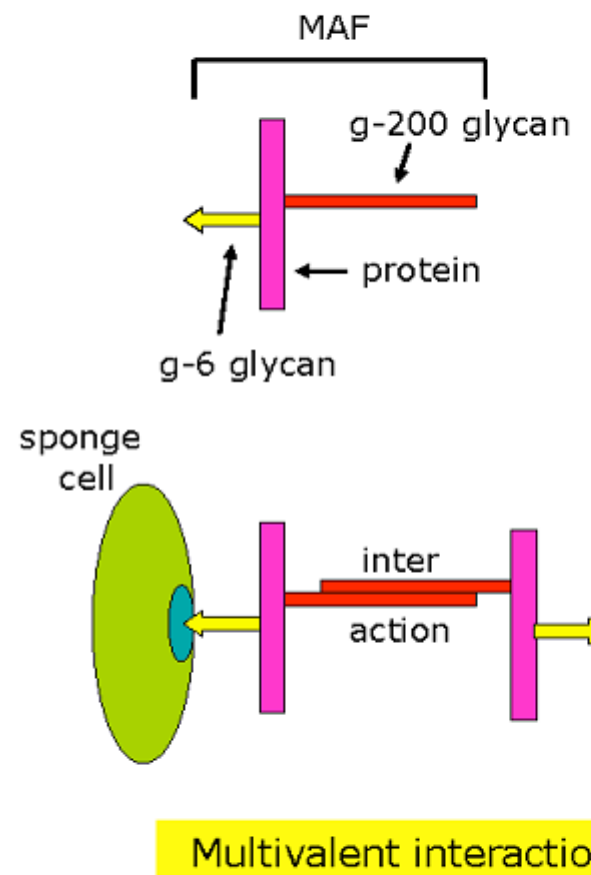
## Proposed model for MAF-mediated sponge cell ad

### *Microciona prolifera*

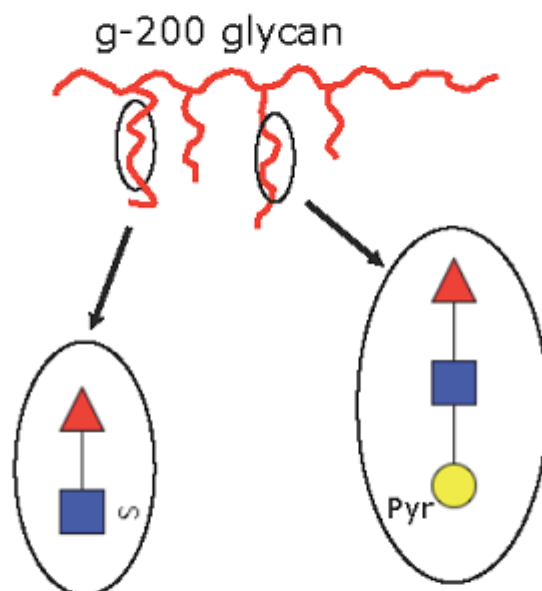
Ca<sup>2+</sup>-independent adherence of g-6 glycans to cell-surface receptors (carbohydrate-protein interaction).

Ca<sup>2+</sup>-dependent self-association of g-200 glycans (carbohydrate-carbohydrate interaction).

Other alkaline earth cations, Mg<sup>2+</sup>, Sr<sup>2+</sup>, Ba<sup>2+</sup> could not replace Ca<sup>2+</sup> as an aggregation-mediating agent. The transition elements Mn<sup>2+</sup> and Cd<sup>2+</sup> could partially replace Ca<sup>2+</sup>.



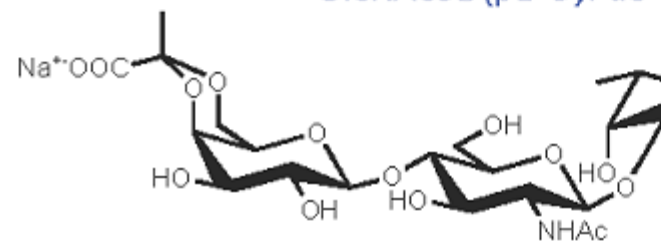
## Structural analysis of MAF fragments



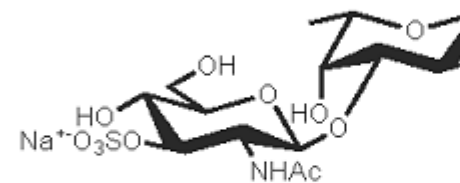
■ = D-GlcNAc    ▲ = L-Fuc    ● = D-Gal

Two oligosaccharide fragments from *M. prolifera* MAF g-200 by reductive hydrolysis were shown to be epitopes for the MAF self-recognition (Mab structure):

- Gal4,6(R)Pyr(β1-4)GlcNAc(β1-3)Fuc
- GlcNAc3S(β1-3)Fuc



Reactive towards the cell surface blocking antibody Block 1

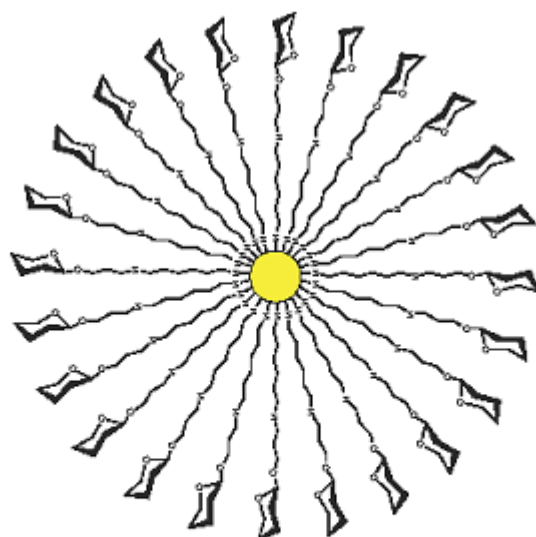


Reactive towards the cell surface blocking antibody Block 2

## Carbohydrate-carbohydrate interactions

Multivalency to overcome low affinity

Gold glyconanoparticles



NMR

TEM

UV

SPR

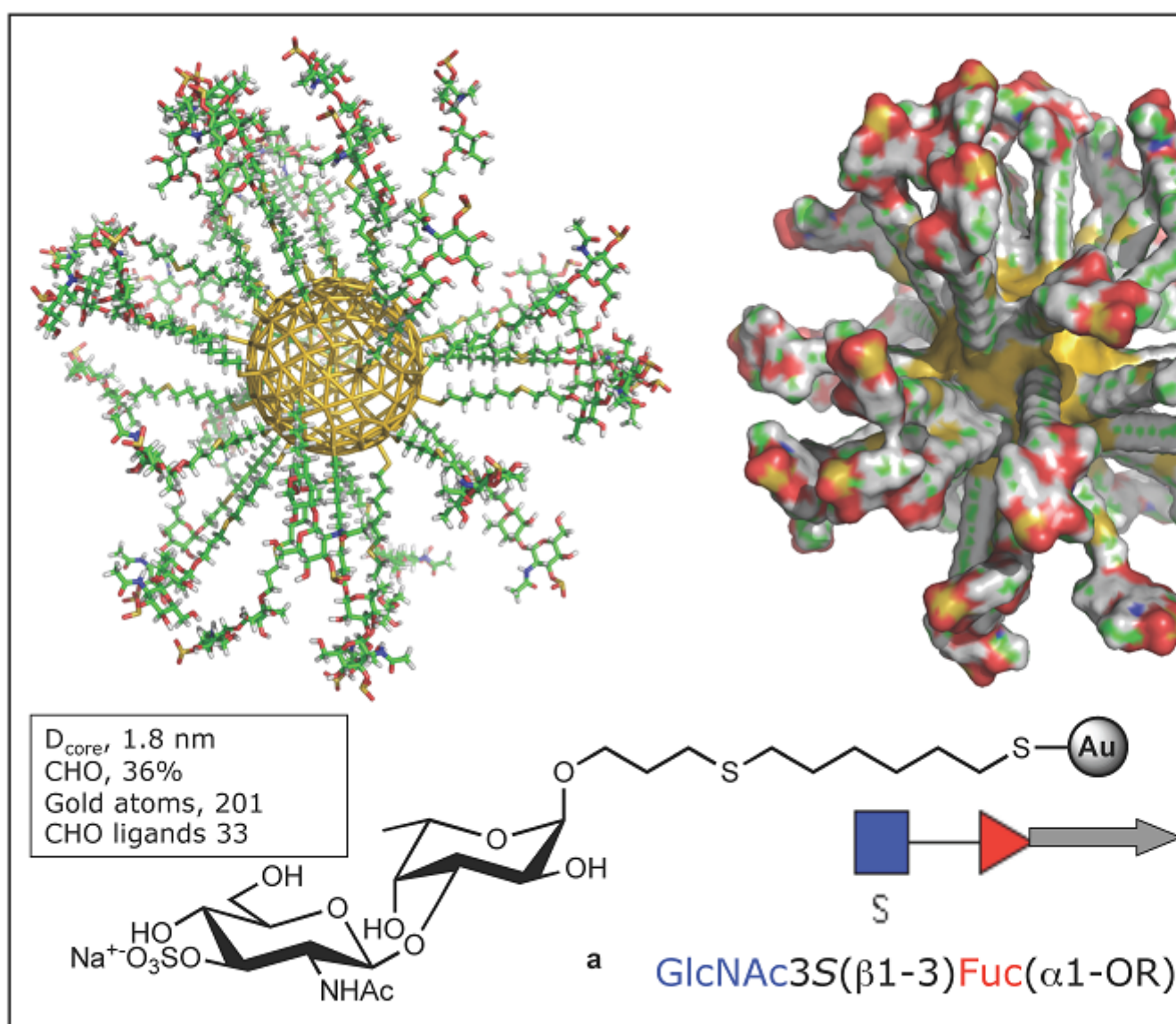
AFM

Neoglycoprotein



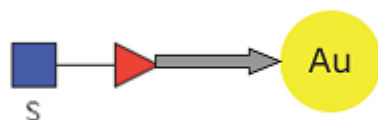
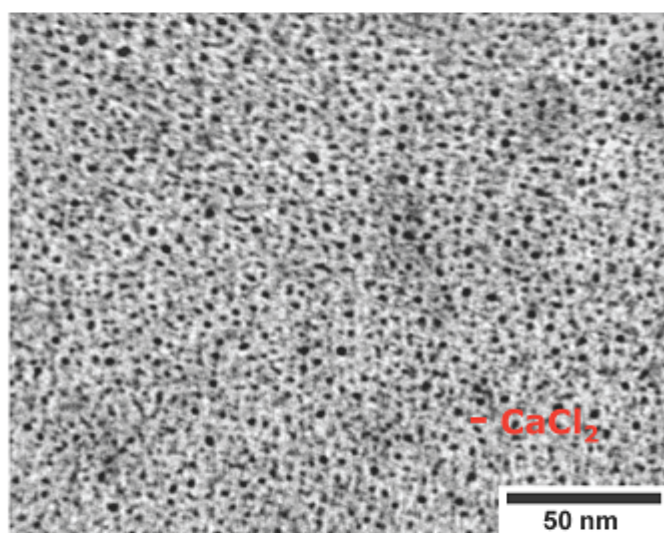
Gold glyco



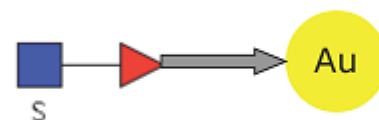
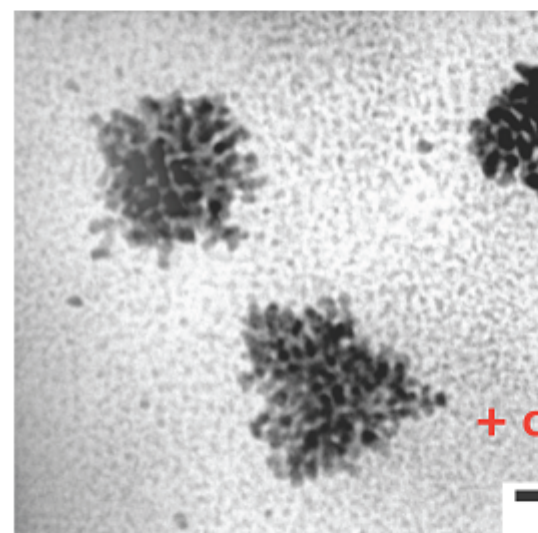




## Transmission Electron Microscopy of gold glyconanoparticles



0.1 mg/ml; **without**  
calcium ions



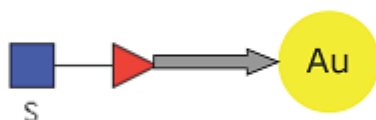
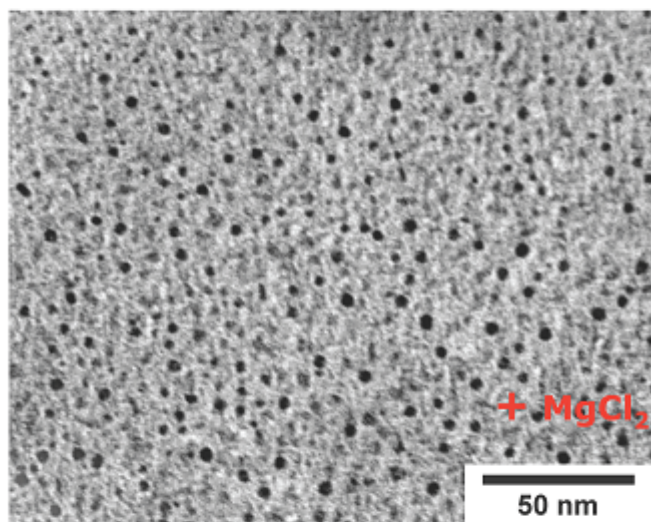
0.1 mg/ml; **with**  
calcium ions (10 m



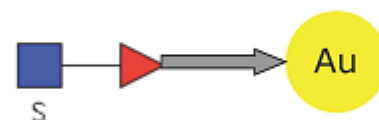
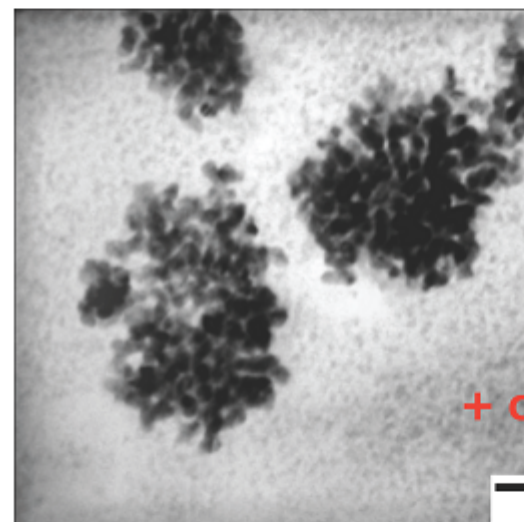
## TEM analysis of gold glyconanoparticles

No self-recognition in the presence of  $\text{MgCl}_2$  and  $\text{MnCl}_2$ .

Weak self-recognition in the presence of  $\text{CdCl}_2$ .



0.1 mg/ml; **with**  
magnesium ions (10 mM)



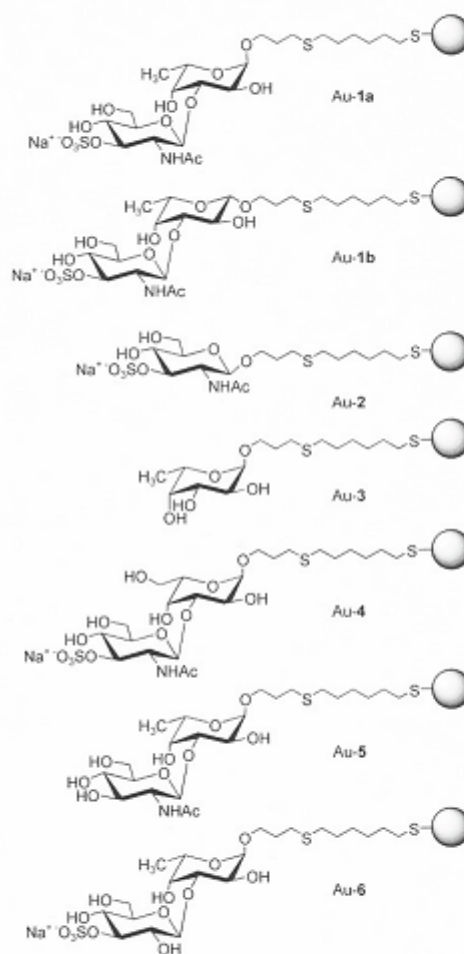
0.1 mg/ml; **with**  
calcium ions (10 mM)

## Gold Glyconanoparticles as Probes to Explore the Carbohydrate-Mediated Self-Recognition of Marine Sponge Cells

Adriana Carvalho de Souza,<sup>[a]</sup> Koen M. Halkes,<sup>[a]</sup>  
 Johannes D. Meeldijk,<sup>[b]</sup> Arie J. Verkleij,<sup>[b]</sup>  
 Johannes F. G. Vliegthart,<sup>[a]</sup> and  
 Johannes P. Kamerling<sup>\*(a)</sup>

Cell aggregation in the red-beard marine sponge *Microciona prolifera* is mediated by a  $2 \times 10^4$  kDa proteoglycan-like macromolecular aggregation factor (MAF), and is based on two highly polyvalent functional properties; a  $\text{Ca}^{2+}$ -dependent proteoglycan self-interaction and a  $\text{Ca}^{2+}$ -independent cell-binding activity.<sup>[1-3]</sup> MAF, the first circular proteoglycan described, is composed of two *N*-glycosylated proteins, MAFp3 and MAFp4, with twenty units of each glycoprotein forming the central ring and the radiating arms, respectively. Each MAFp3 carries one or two copies of a 200 kDa acidic glycan, g-200, whereas each MAFp4 carries about 50 copies of a 6 kDa glycan, g-6.<sup>[1]</sup> The MAFp4 arms of the sunburst-like proteoglycan are linked to cell-surface binding receptors, while the MAFp3 ring exposes the g-200 glycans so that they can engage in the  $\text{Ca}^{2+}$ -dependent self-association (for a detailed review, see ref. [4]). By making use of MAF-specific monoclonal antibodies, it could be demonstrated that the self-association of MAF occurs through highly repetitive epitopes on the g-200 glycan.<sup>[5,6]</sup> One of these epitopes was shown to be the sulfated disaccharide  $\text{GlcNAc}3\text{S}(\beta 1-3)\text{Fucp}$ .<sup>[7]</sup> To gain insight into the role of carbohydrate interactions in MAF self-aggregation, we designed a challenging system for mimicking the g-200 self-association.<sup>[8]</sup> By using the synthetic sulfated disaccharide, multivalently presented as a bovine serum albumin conjugate, and surface plasmon resonance spectroscopy, it was shown that  $\text{Ca}^{2+}$ -dependent carbohydrate self-recognition is a major force in the g-200 association phenomenon.

Gold glyconanoparticles have been successfully used as inert multivalent systems to explore either carbohydrate self-interac-



Scheme 1. Gold glyconanoparticles Au-1 a/b to Au-6, related to the MAF sulfated disaccharide epitope.



Adriana  
de Souza

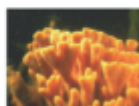
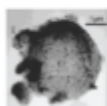
ChemB  
6 (200

### Carbohydrate-carbohydrate interactions

#### Marine sponges

1998-2009

- Primitive models for studying cell-cell recognition
- Species-specific aggregation of cells



Microscopic analysis

- When placed in artificial sea water free of  $Ca^{2+}$  ions, a **complete dissociation** of the sponge tissue into a suspension of cells occurs.
- Formation of **cell-aggregation** by adding  $Ca^{2+}$  to the cell suspension; completely functional sponges can be the result.
- Cell surface proteoglycans underlie the  $Ca^{2+}$ -dependent molecular mechanism of the self-recognition; coding: **aggregation factors (AF)**.
- Sponge species specificity of the aggregation process; **coarse-specific** sponge cell sorting. Can be mimicked by different: coloured beads, coated with species-specific AFs.

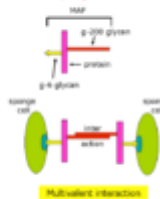
reposed model for MAF-mediated sponge cell adhesion

*Sciona prolifera*

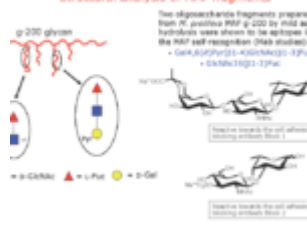
$Ca^{2+}$ -independent adherence of  $\alpha$ -D-glucans to cell-surface receptors (carbohydrate-ribonucleic interaction).

$Ca^{2+}$ -dependent self-association of  $\beta$ -D-glucans (carboxylate-carboxylate interaction).

Two alternate ways (cation,  $Mg^{2+}$ ,  $Ba^{2+}$ ,  $Ca^{2+}$ ) could still replace  $Ca^{2+}$  as cell aggregation mediating agent. The electron densities  $Mg^{2+}$  and  $Ca^{2+}$  would likely replace  $Ca^{2+}$ .



Structural analysis of MAF fragments



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[hans37bis.png](#)

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[hans38bis.png](#)

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[hans40bis.png](#)

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## Category

1. News