

Hyaluronic acid dipeptide gels studied by Raman Spectroscopy, Atomic Force Microscopy and DFT Calculations



COMPUTATIONAL
CHEMISTRY
DAY 2025

07. lipnja 2025.

Vlasta Mohaček Grošev

Jože Grdadolnik



Gelovi – razni mehanizmi i svrhe

- ▶ Gelovi koji pomažu zacijeljivanju rana

Molekule se slobodno umrežavaju s polimerom nosača i ekstracelularnom matricom

Npr u gelu Bepanthene 4in1

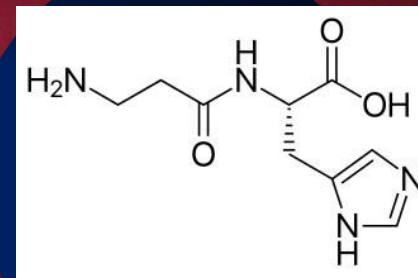
- ▶ Gelovi koji otpuštaju aktivne tvari

Npr Amfostine za djelovanje u probavnom traktu nakon želuca, zaštićen gelom da izdrži kiseli pH

- ▶ Antibakterijski gelovi za čišćenje ruku

90% su alkoholni

- antibakterijski gelovi (alkoholni)
- gelovi koji ubrzavaju zacijeljivanje



AKTIVNA SUPSTANCA JE DIPEPTID
KARNOZIN

- API, active pharmaceutical ingredient (API) – ne bi se trebao kemijski vezati za polimer u gelu ako je namijenjen vezanju za ciljani enzim ili sl.

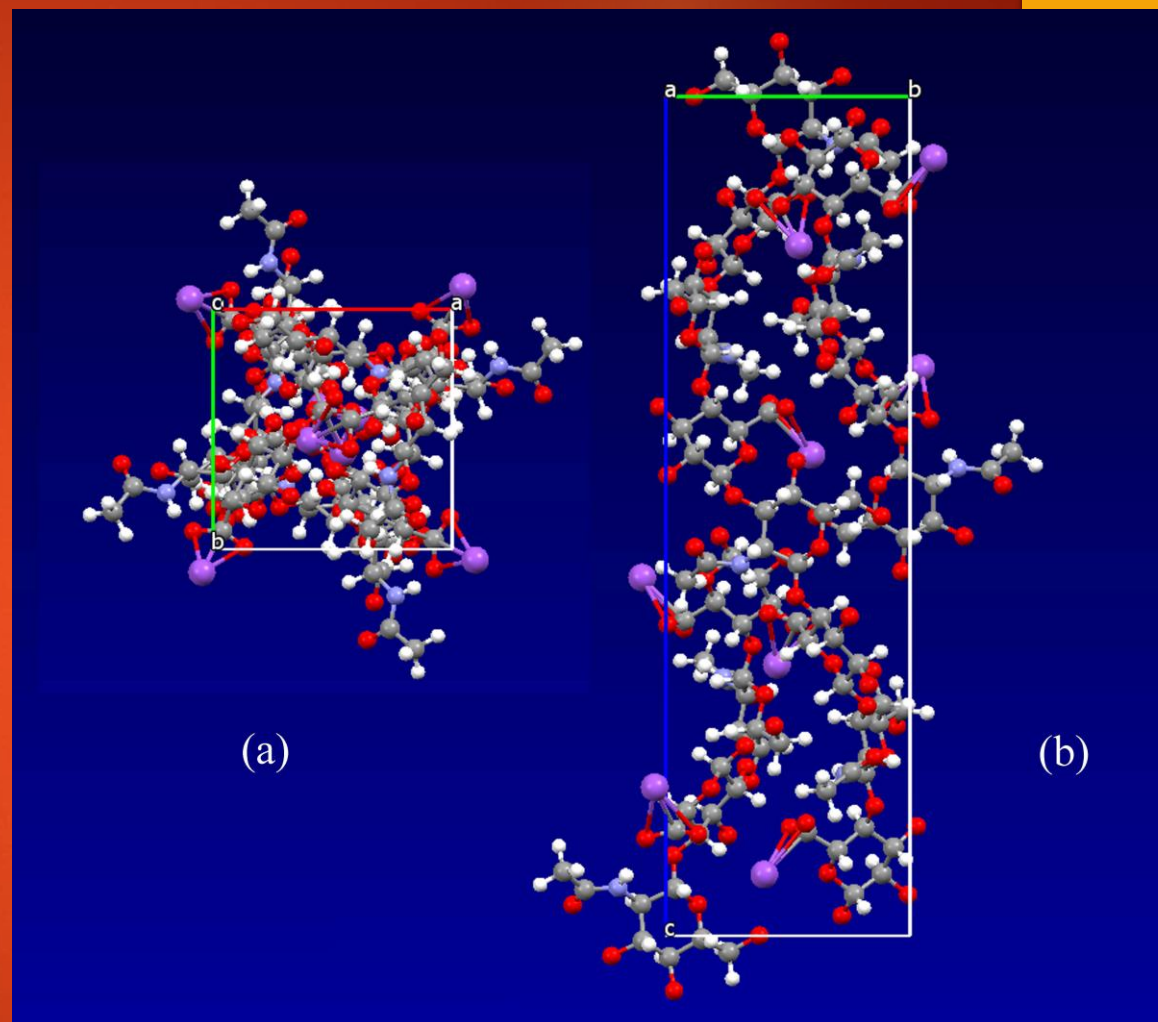


- U slučaju vezanja za polimer u gelu, dipeptid može poslužiti kao sredstvo za poticanje zacijeljivanja rana



Hijaluronska kiselina

Molarna masa 700 000 g/mol

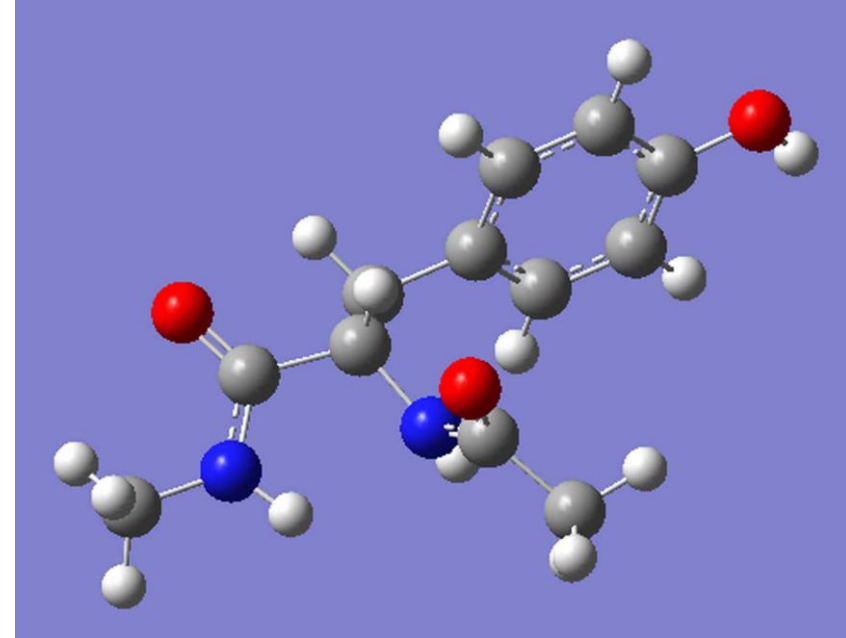
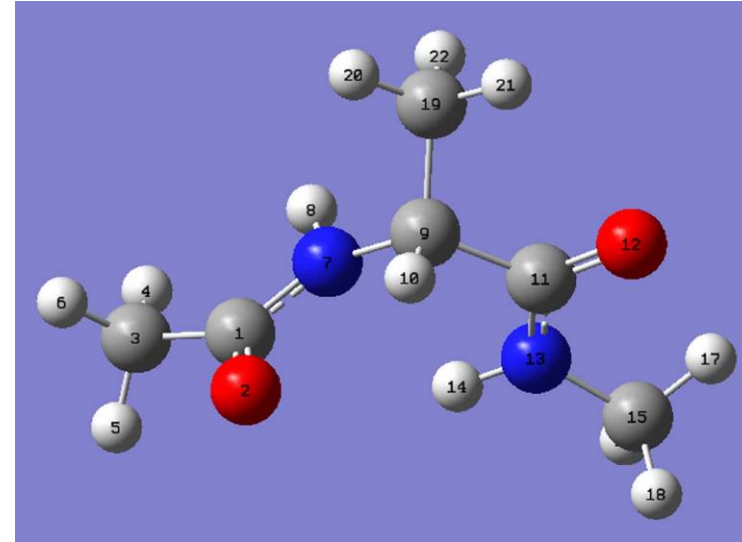


Odredili Guss et al. *J. Mol. Biol.* **95** (1975) 359-384.
Slika pripremljena s *Mercury* programom.

N-acetil-alanin-metil amid

NAcAlaNHMA

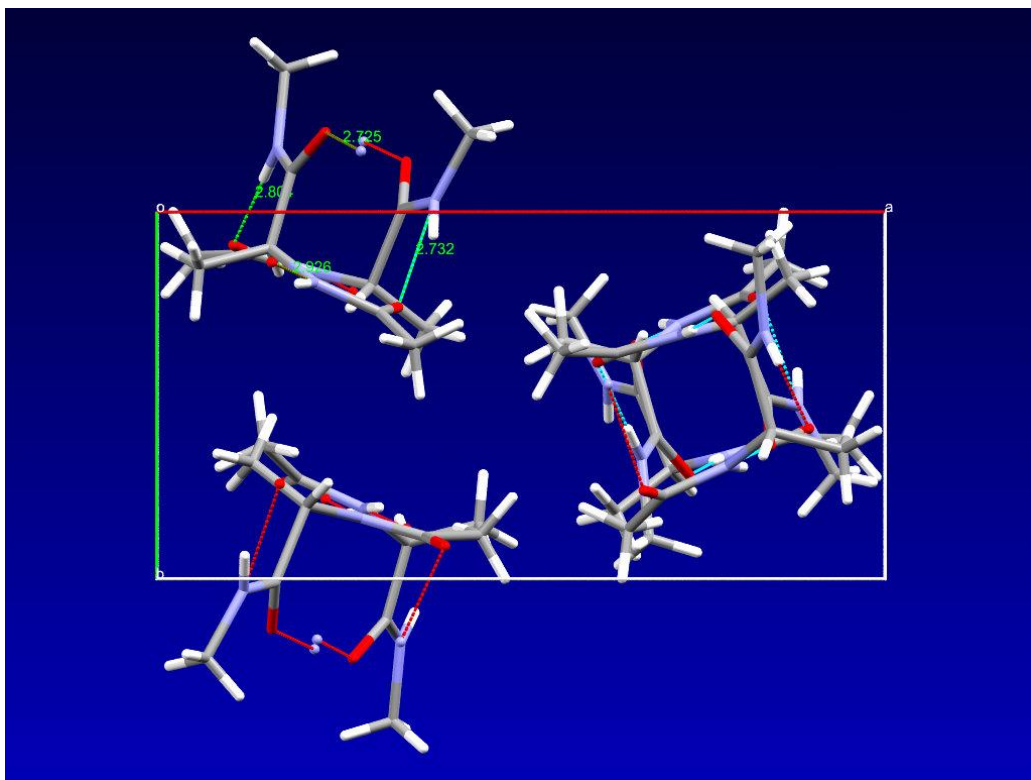
N-acetil-tirozin-metil amid
NAcTyrNHMA



Kristalne strukture dipeptida

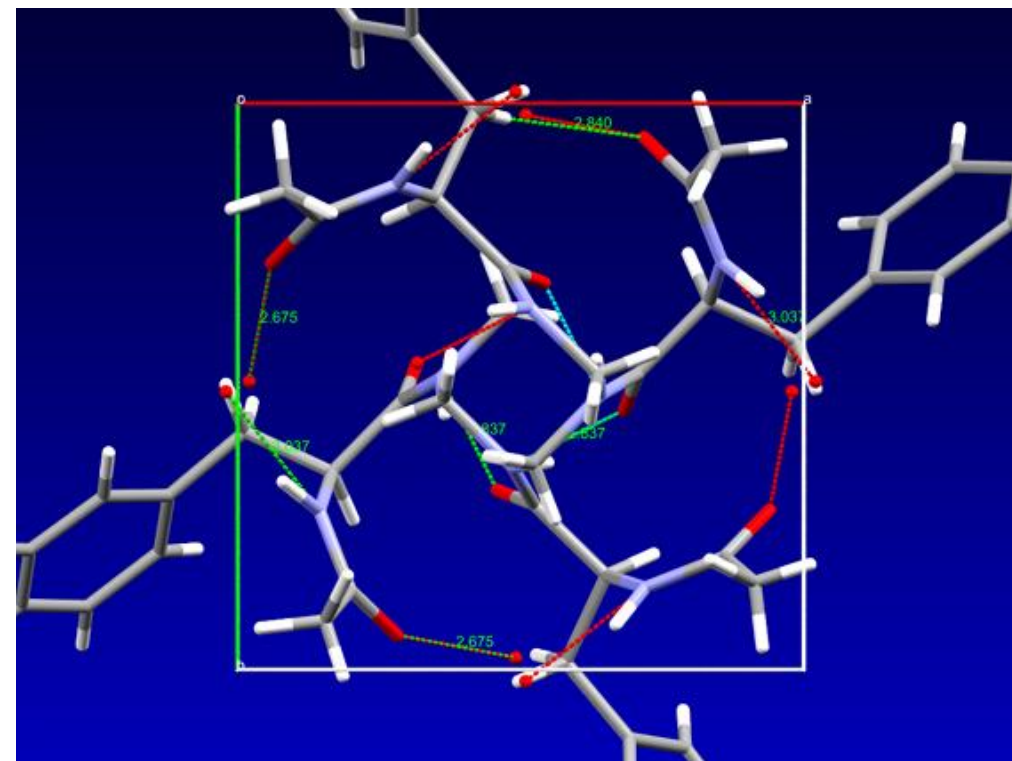
NAcAlaNHMA

Harada & Itaka, *Acta Cryst B* 30 (1974) 1452.



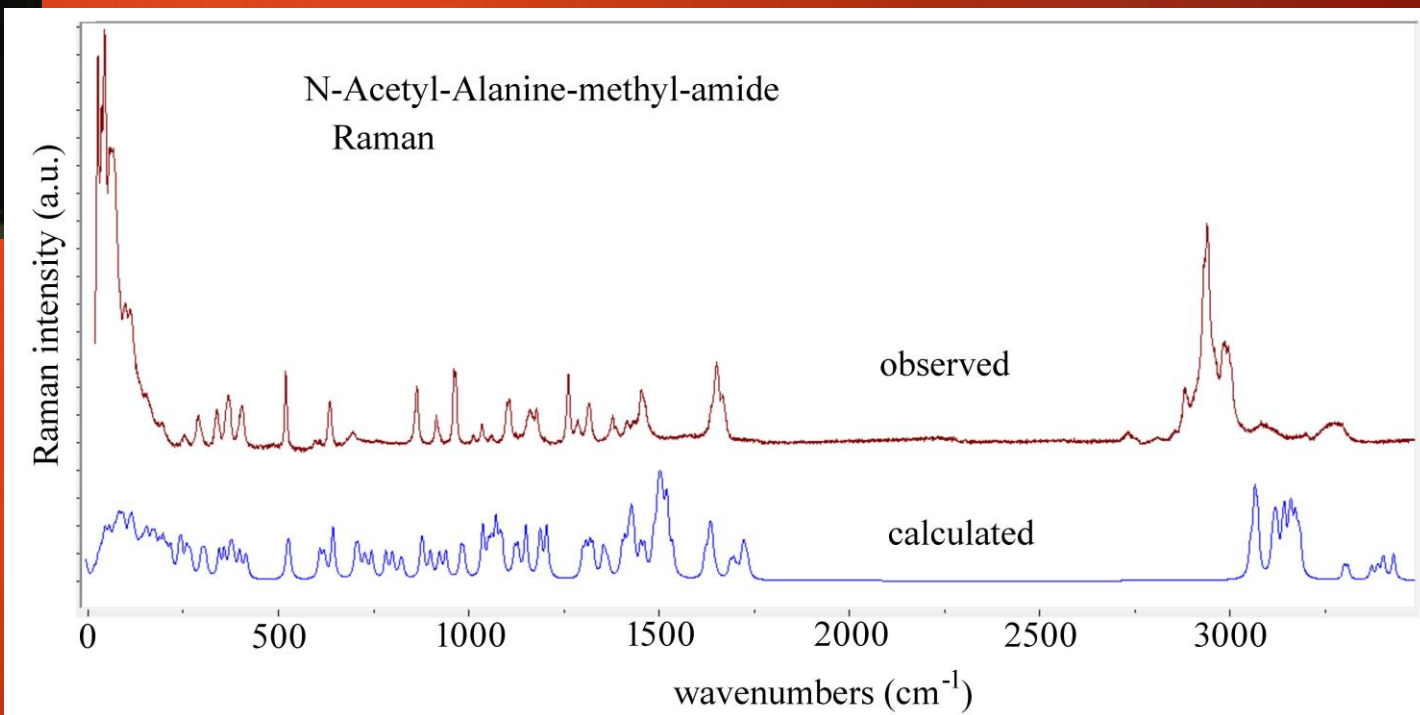
NAcTyrNHMA

Cotrait & Bidaeu, *Acta Cryst B* 30 (1974) 1024.



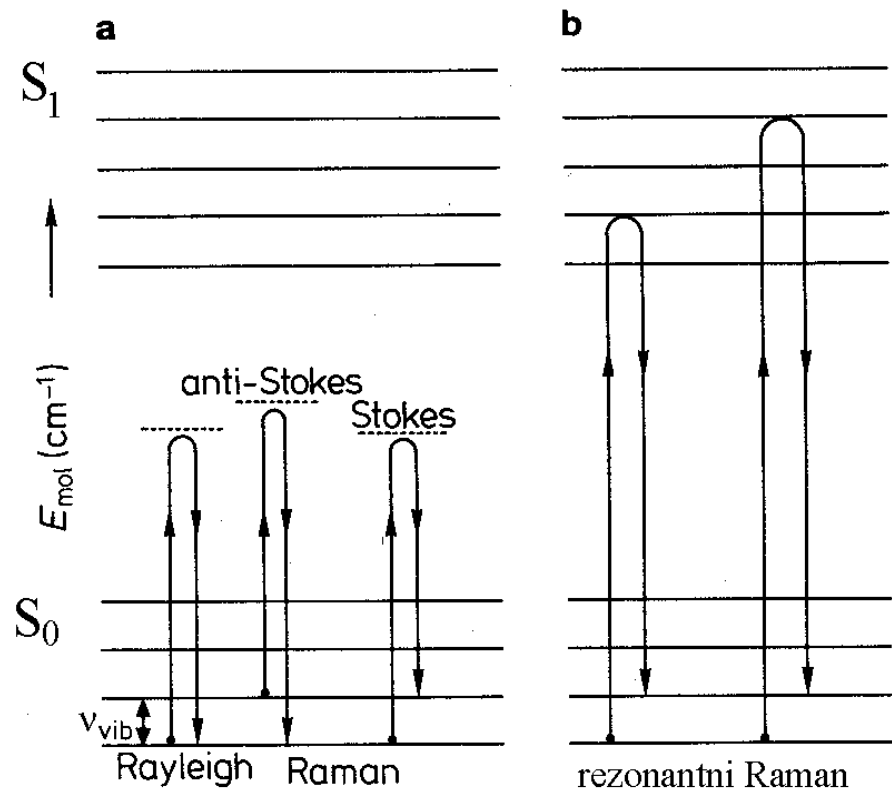


Horiba JobinYvon T64000
Ramanov spektrometar s
tri rešetke za snimanja
 $10 - 4000 \text{ cm}^{-1}$

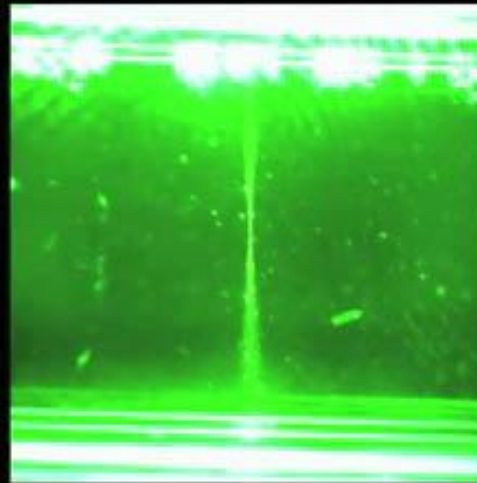


Ramanova spektroskopija

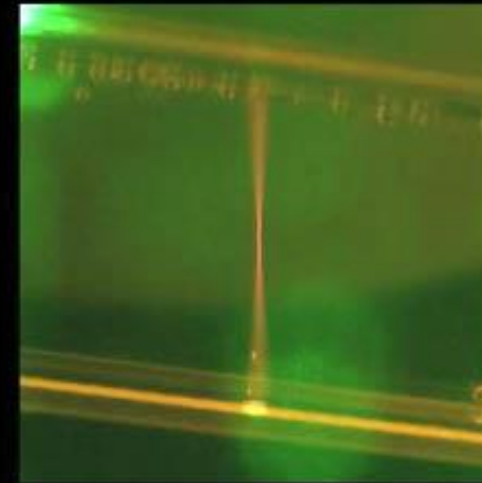
<https://youtu.be/G1jXR5PhUc0>



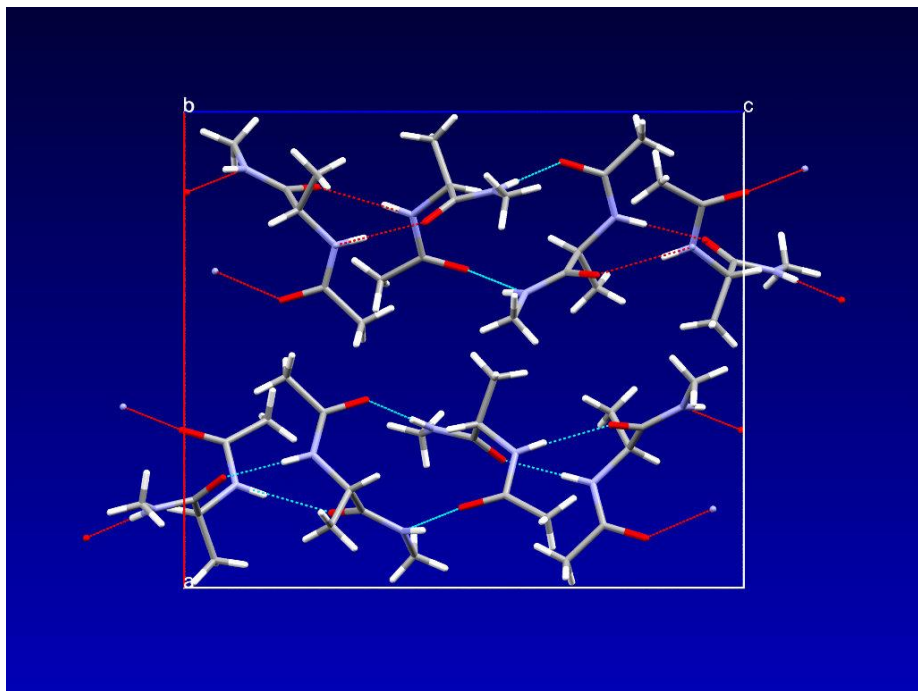
Rayleigh scattered light



Raman scattered light



Izračunati fononski spektar NAcAlaNHMA



$P2_1 2_1 2_1$
 $Z = 4$

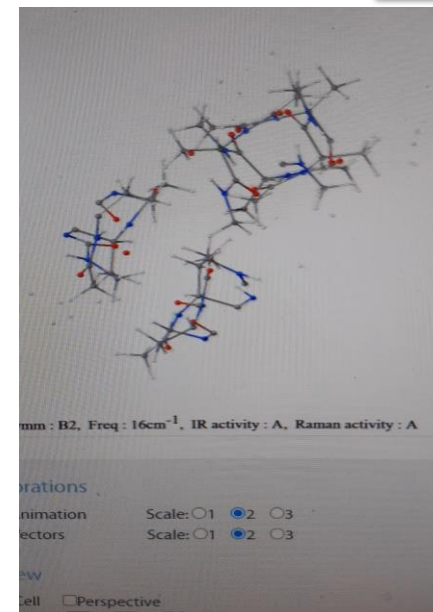
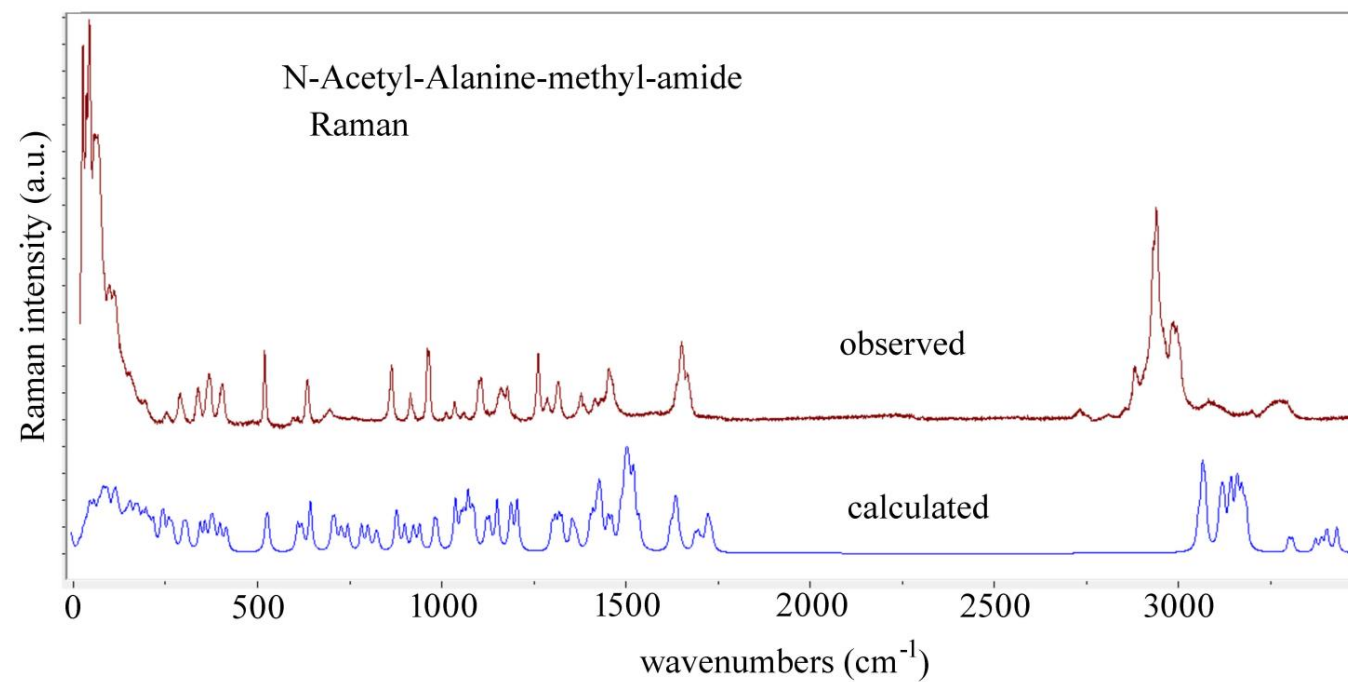
Harada & Itaka, *Acta Cryst B* **30** (1974) 1452.

Teorijski ramanski spektar: CRYSTAL09

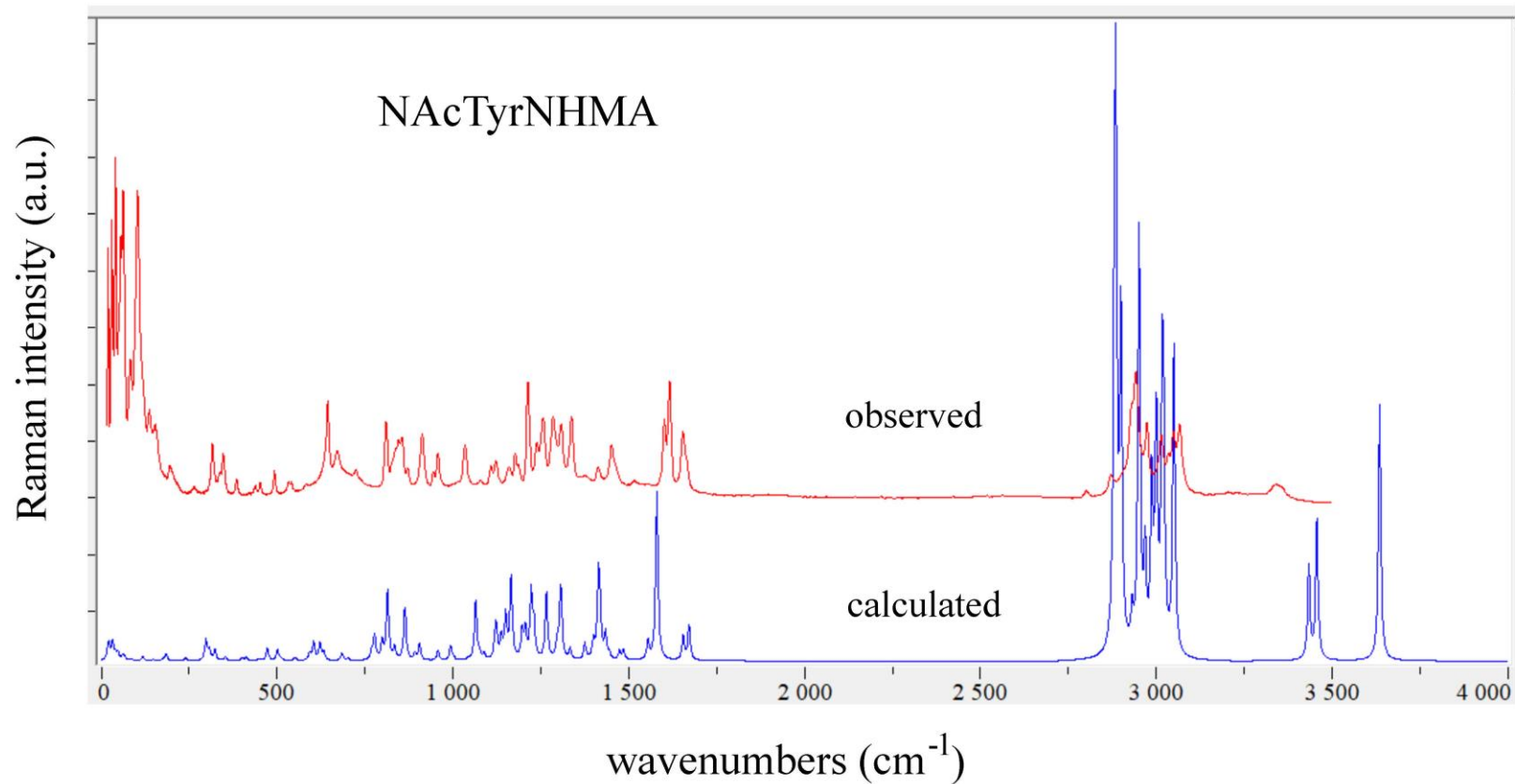
Protone je u LAANMA cif
datoteku dodao dr.
K.Molčanov

DFT
B3LYP
XLGRID
CHUNKS
200
END
SHRINK
0 0
2 2 2
LEVSHIFT
6 1
FMIXING
30
MAXCYCLE
800
TOLINTEG
7 7 7 7 14
NOBIPOLA
SCFDIR
ENDSCF

Teorijski spektar: CRYSTAL09



- Online view of the modes:
- https://crysplot.crystalsolutions.eu/web_pages_yves3/vibration.html



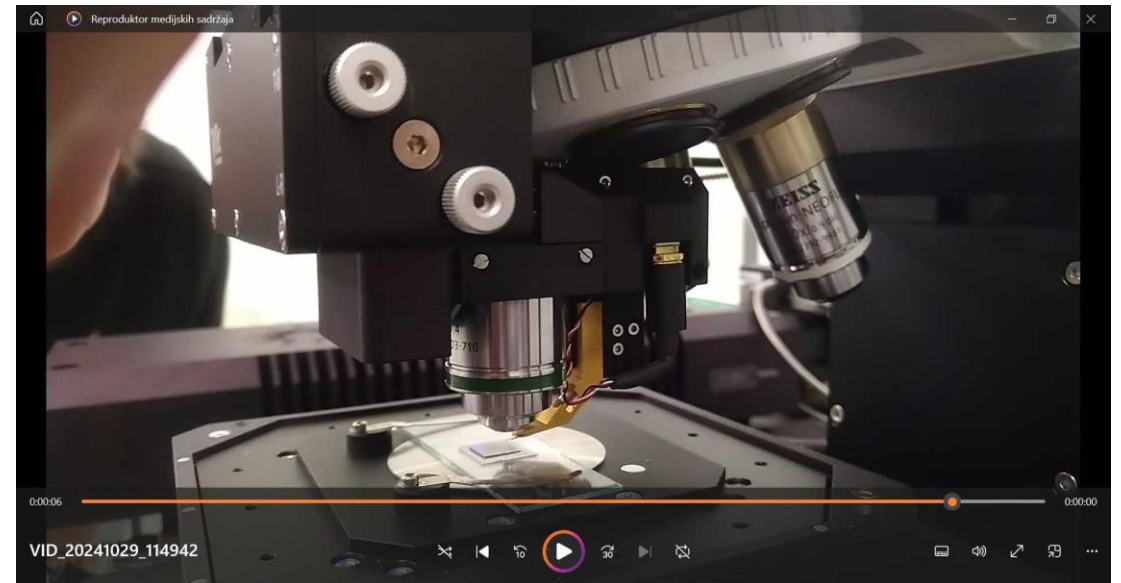
Opaženi spektar: T64000 HJY

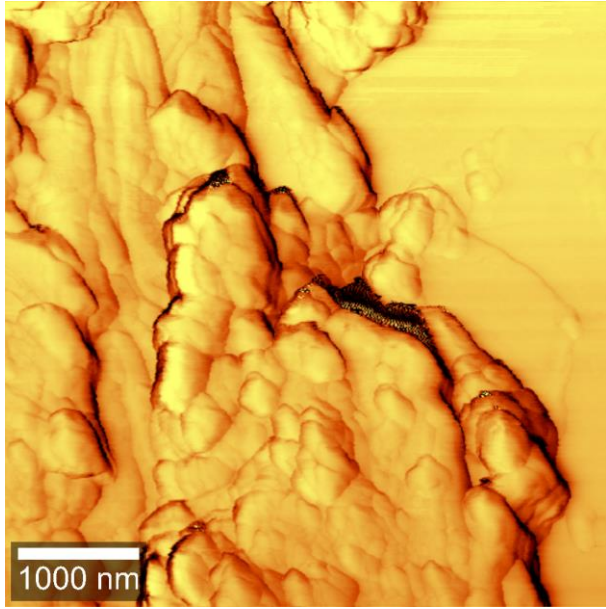
Teorijski spektar: Gaussian16, B3LYP, 6-31++G(d,p), faktor skaliranja 0.9497

Table 1. Comparison of the most important observed Raman (R) (Figure 3.) and infrared (IR) (Figure 4) bands exhibiting intermolecular hydrogen bonding in NAcAlaNHMA and NAcTyrNHMA crystals with assignation.

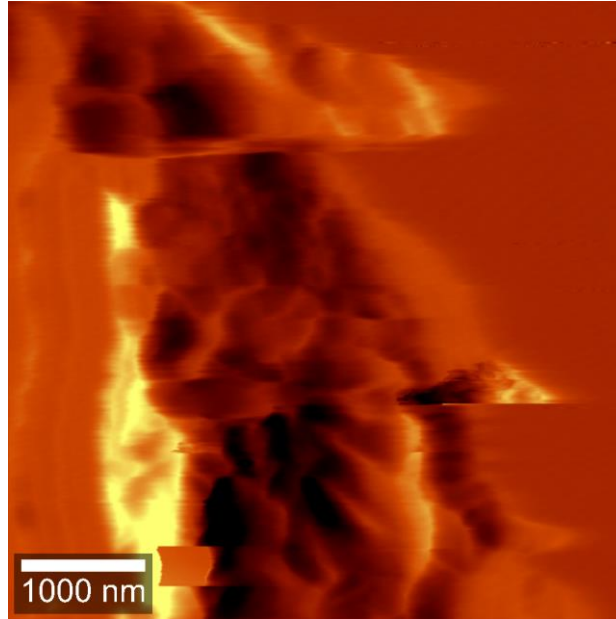
NAcAlaNHMA	NAcTyrNHMA	assignment
1667 R, 1664 IR		Amide I
1651 R	1649, 1636 IR	Amide I
1639 R, 1636 IR		Amide I
1578 R		Amide II
1567 R, 1553 IR	1556 R	Amide II
1533 R	1532, 1514 R	Amide II
	1462 R	Amide II
1262 R, 1259 IR	1237 R, 1235 IR	Amide III
	1121 R, 1120 IR	$\delta(\text{C-OH})$
1106 R, 1103 IR		$\nu(\text{C-N}) + \delta(\text{CH}_3)$
	1033 R, 1034 IR	$\nu(\text{C-N})$
635 R, 634 IR		$\delta(\text{O=C-N})$
597 R, 594 IR	580 R, 581 IR	$\delta(\text{O=C-N})$
	541 R, 561 IR	$\gamma(\text{C-N})$
403 R, 399 ¹ IR		$\delta(\text{O=C-N}) + \delta(\text{N-C-C})$
369 R, 366 ¹ IR		$\delta(\text{O=C-N}) + \delta(\text{N-C-C})$
	347 R, 348 IR ²	$\delta(\text{N-C-C})$

Raman-AFM instrument WiTEC alpha 300 RA

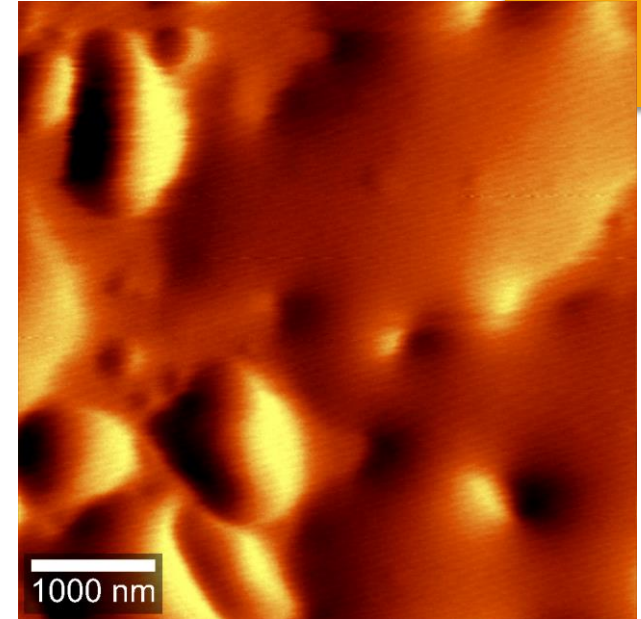




Hyaluronic acid 3.3 wt%



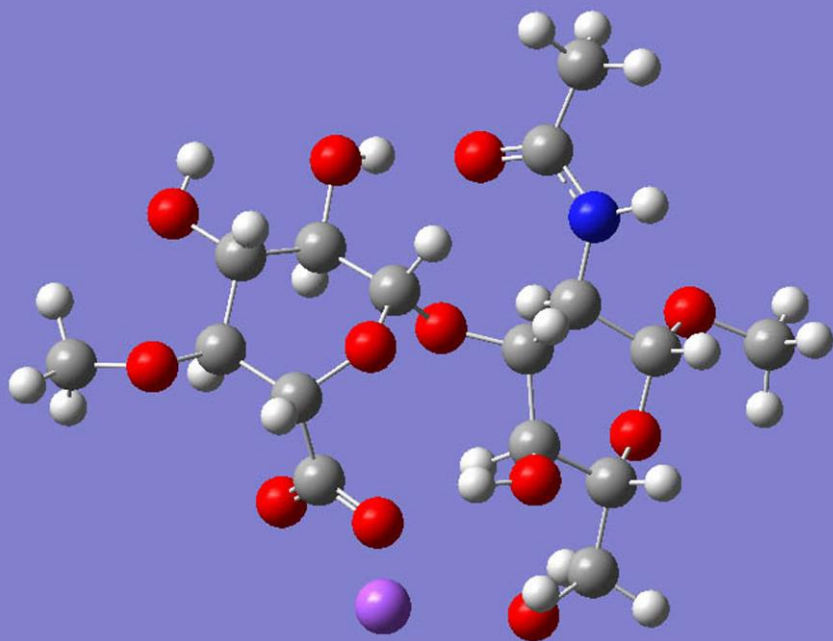
HA + NAcAlaNHMA
0.5 % wt+0.71% wt
98.78% wt water



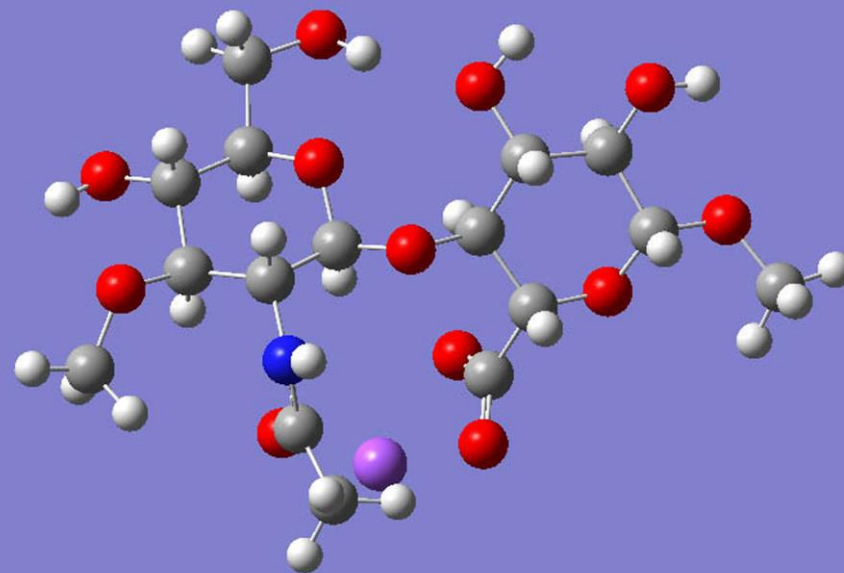
HA + NAcTyrNHMA
0.51% wt +1.16% wt
98.3% wt water

Tapping mode AFM, 75 kHz, 2.8 N/m tip

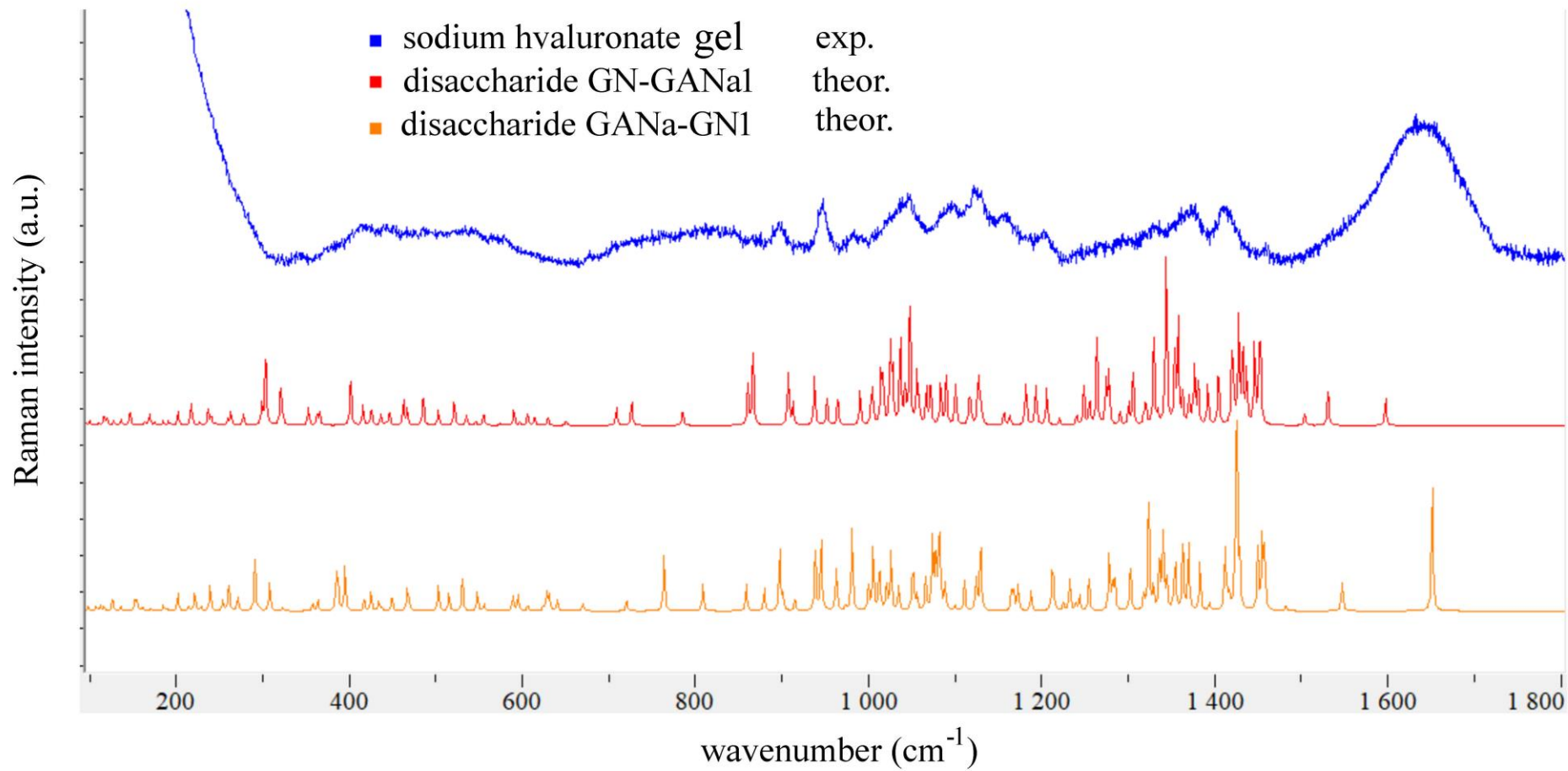
GANa-GN1



GN-GANa1



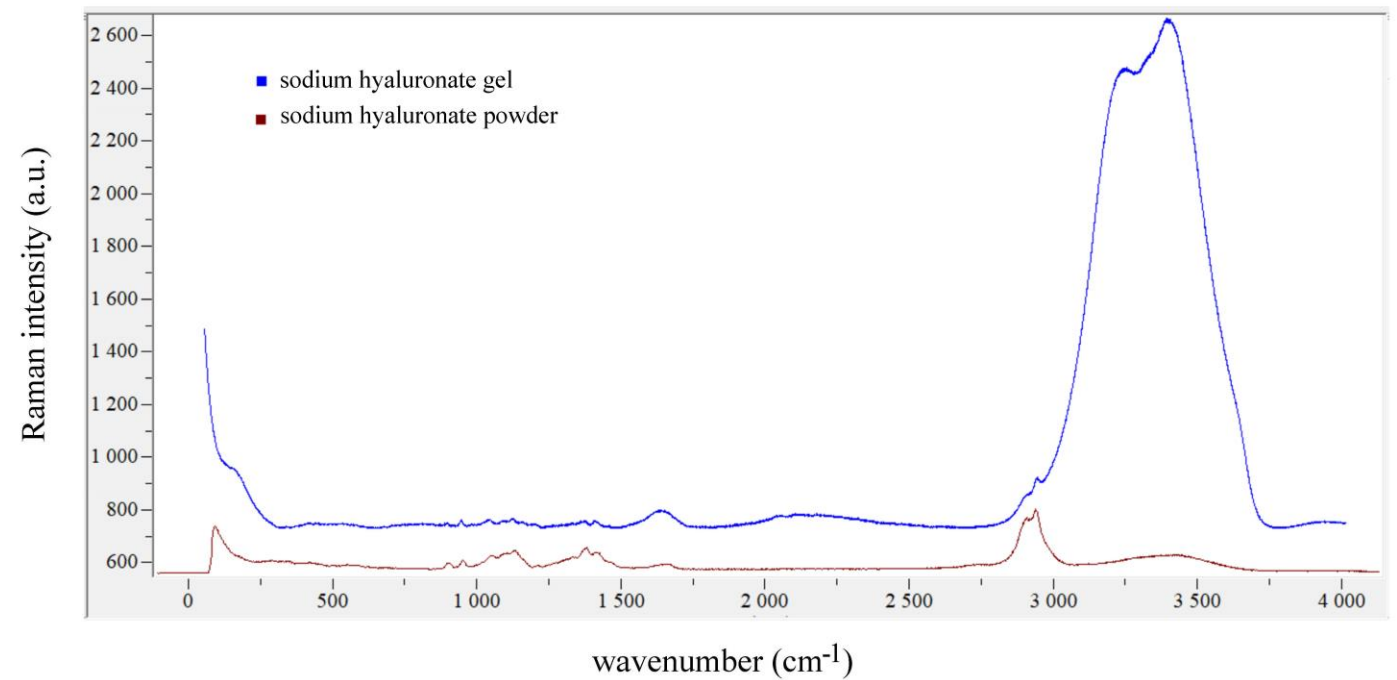
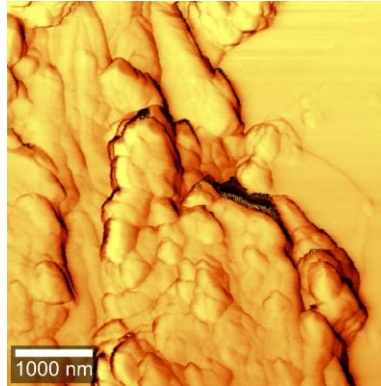
Hijaluronska kiselina je kopolimer glukuronske kiseline (GA) i N-acetil-glukozamina (GN). GA se veže 1-4 glikozidnom vezom na GN, a GN s 1-3 glikozidnom vezom na GA.



Izračunati spektri kopolimernih jedinica: Gaussian16

Gel hijaluronske
kiseline

Ramanski
spektar gela
uspoređen sa
spektrom
praha.



Priprema gelova

Table 2. Mass weighted composition of studied gels

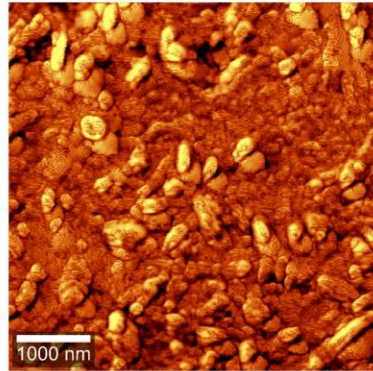
Gel	Wt % HA	Wt%NACAlaNHMA	Wt % NAcTyrNHMA	Wt% water
1	1	0	0	99
2	0.51	0.71	0	98.78
3	0.51	0	1.16	98.3
4	2	0	0	98
5	1.85	7.27	0	90.88
6	1.85	0	7.27	90.88
7	3.8	1.4	0	94.8
8	3.8	0	2.22	94.02

Table 3. Number of molecules of dipeptides and water per one disaccharide unit of HA

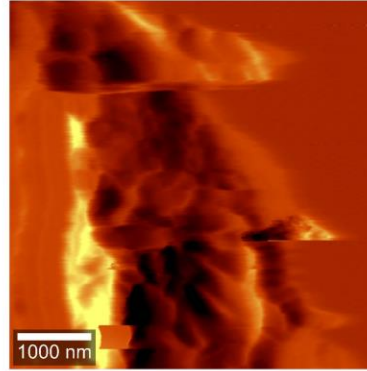
Gel	Unit HA	N molecules NACAlaNHMA	N molecules NAcTyrNHMA	N molecules water
1	1	0	0	2150
2	1	4	0	4300
3	1	0	2	4300
4	1	0	0	1096
5	1	11	0	1096
6	1	0	7	1096
7	1	1	0	558
8	1	0	1	558

Gels

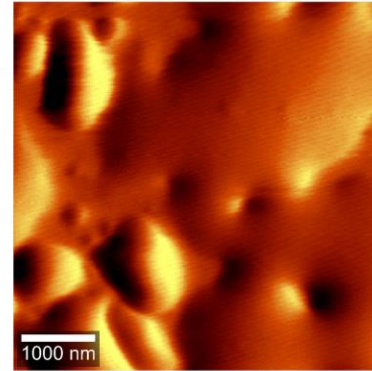
1.



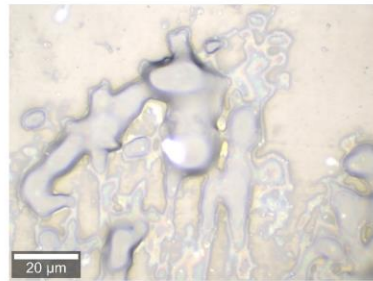
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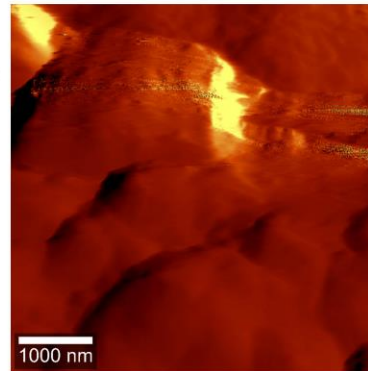
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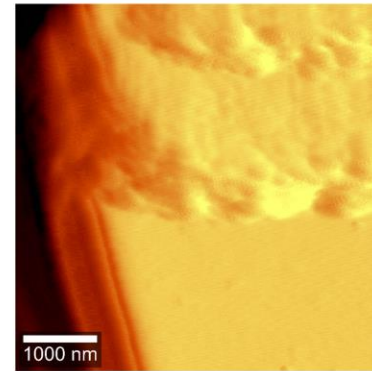
4.



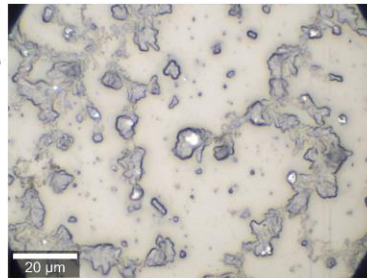
5.



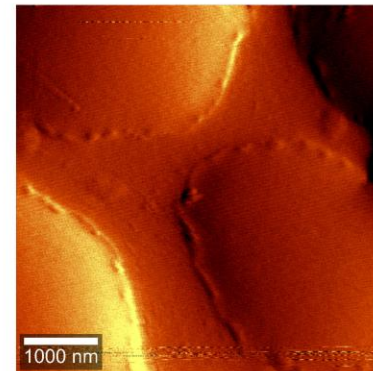
6.



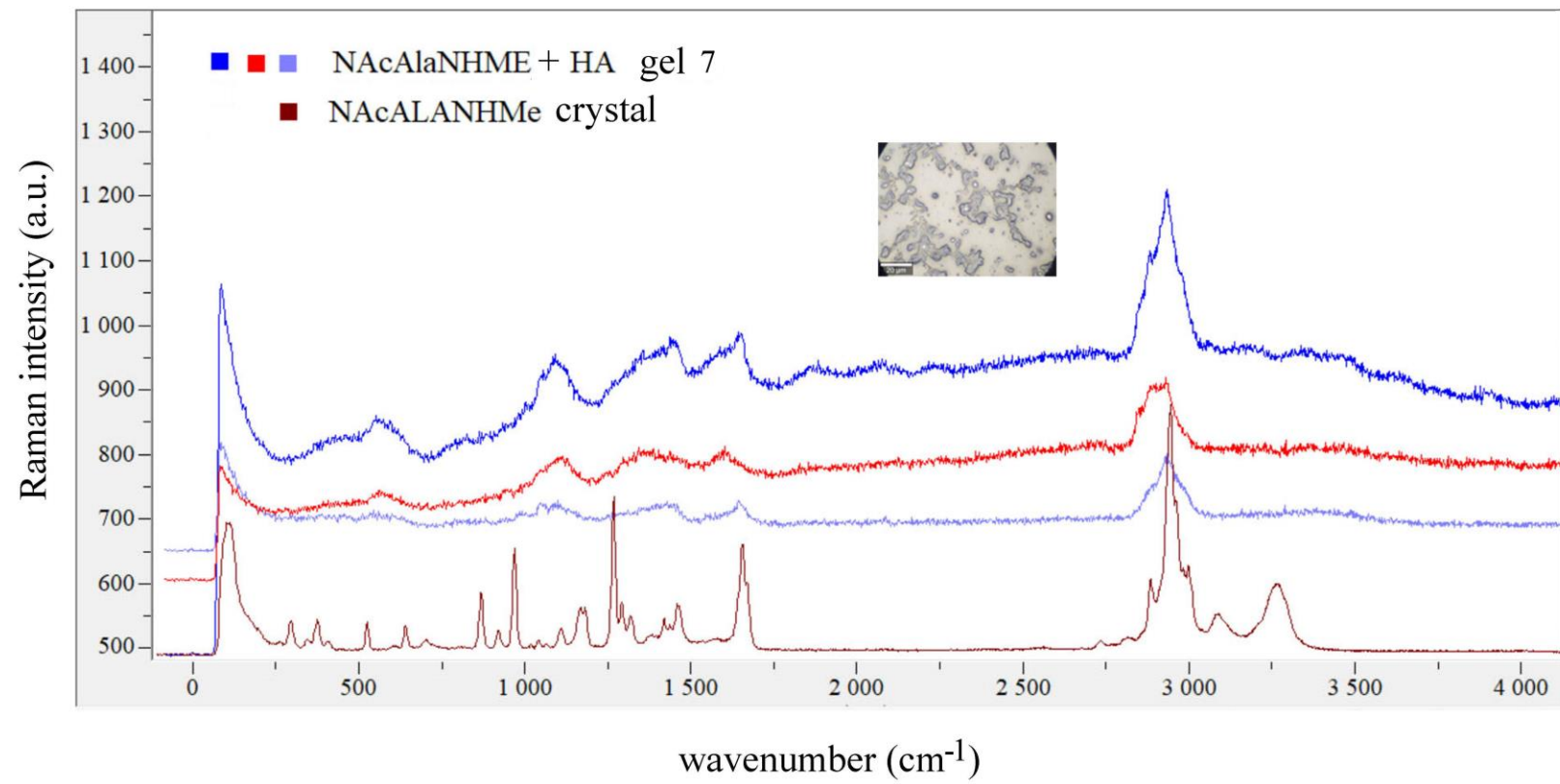
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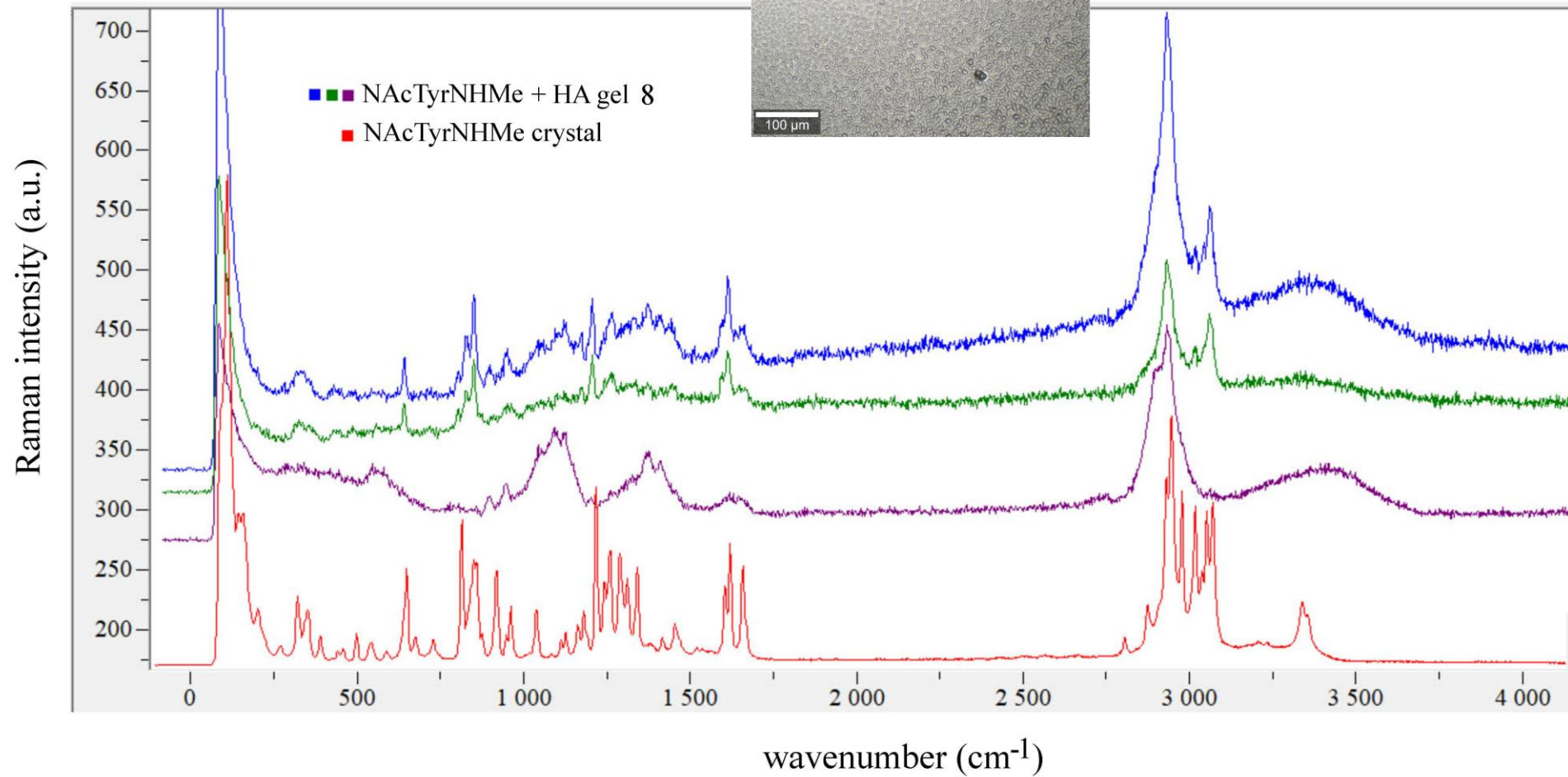
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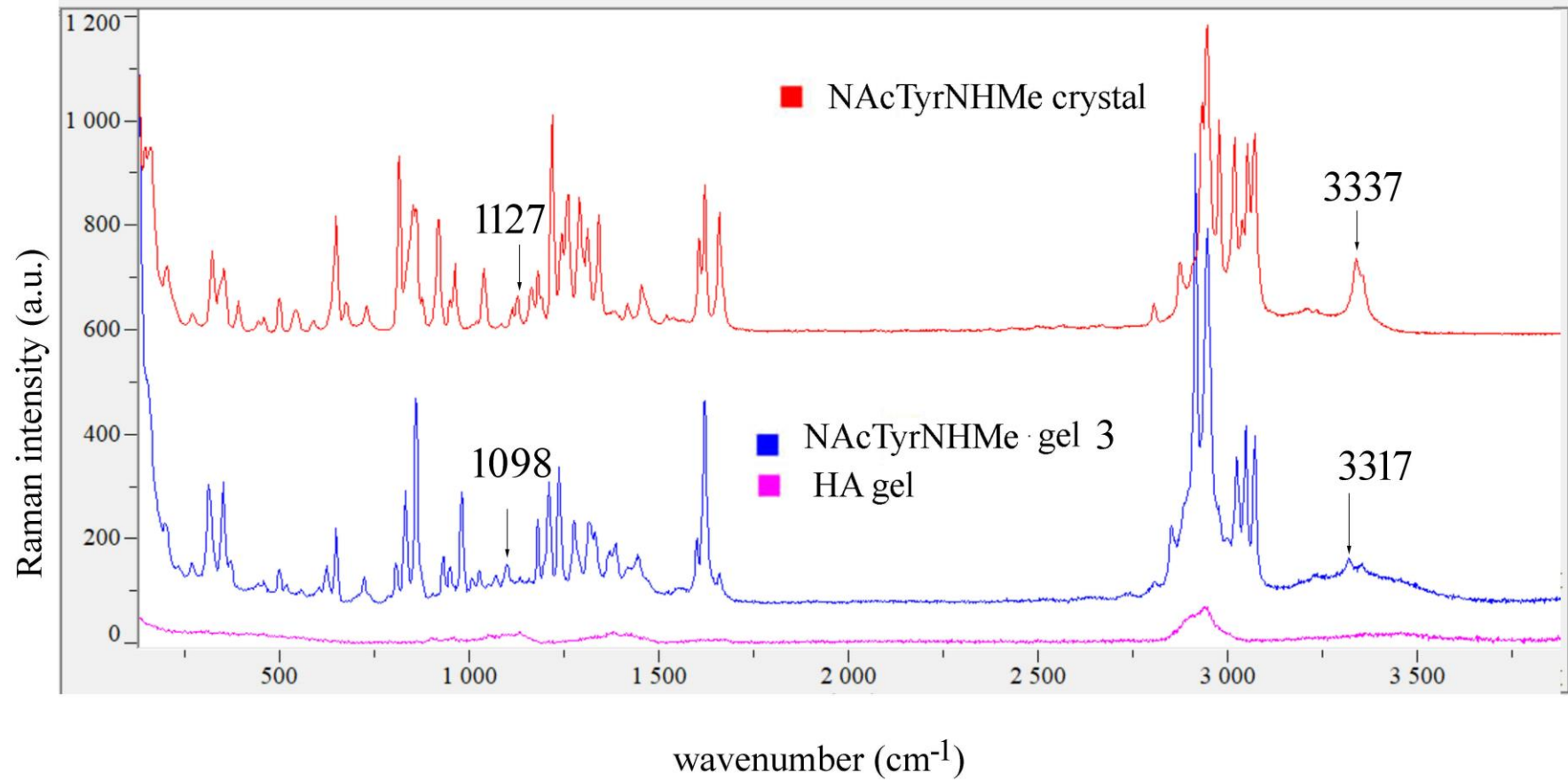
Gel 7

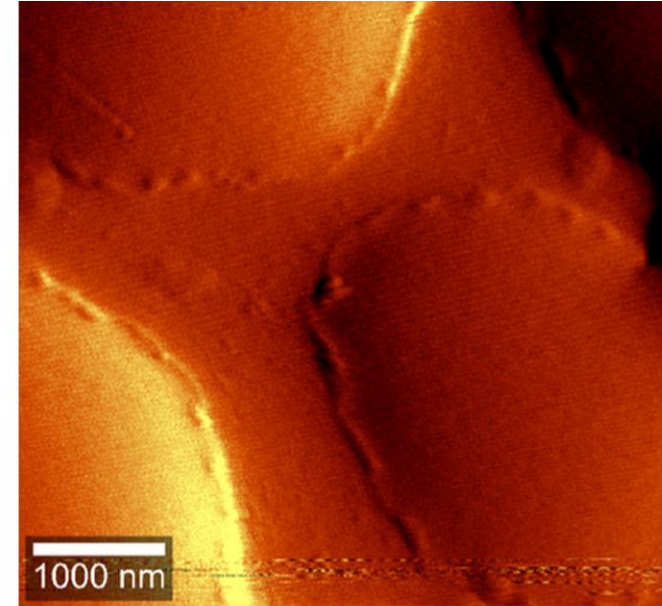
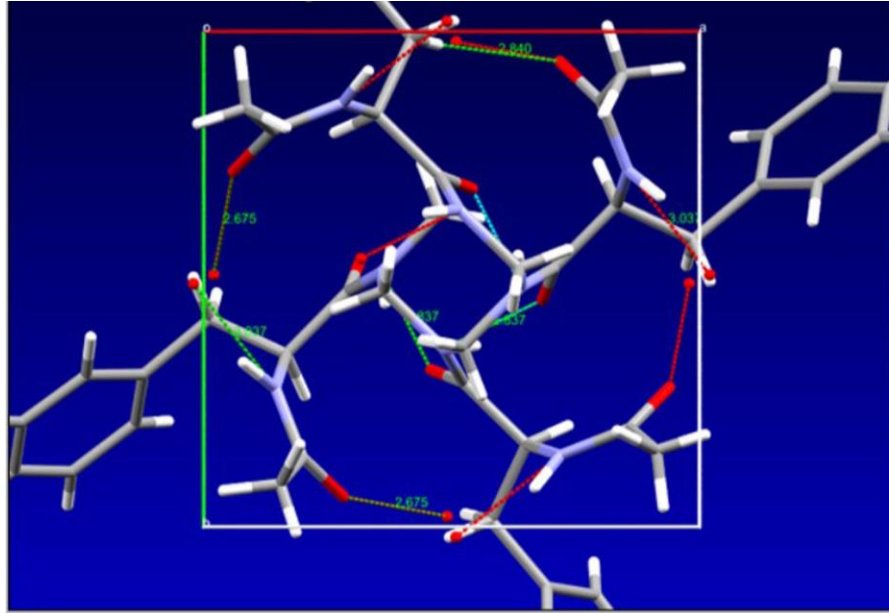


Gel 8



Gel 3





N-Acetyl-Tyrosine-Methyl-Amide binds to hyaluronic acid

Conclusions: While NAcAlaNHMA forms a physical gel with HA, NAcTyrNHMA when mixed with HA in 1:2 ratio can form a chemical gel.

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by Vlasta Mohaček-Grošev* and Jože Grdadolnik

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Article

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Hvala na pažnji!