

APPENDIX A: Precleaning protocol for QSensor QSX 310 T (Biolin Scientific)

1. Immerse the sensor surfaces in 1% Hellmanex II for 30 minutes at room temperature.
2. Rinse with milliQ water.
3. Dry with nitrogen gas.
4. Sonicate in 99% ethanol for 10 minutes.
5. Rinse with milliQ water.
6. Dry with nitrogen gas.
7. UV/ozone treat for 10 minutes

APPENDIX B: Calculation of LL-37 functions and ratios

1) Elemental composition of LL-37 (LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLPRTES)

Amino acid	Abbreviation	Numb	Numb atoms in a.a. residue					Numb of atoms in LL-37				
			C	O	N	S	H	C	O	N	S	H
alanine (Ala)	A	0	3	1	1	0	5	0	0	0	0	0
arginine (Arg)	R	5	6	1	4	0	12	30	5	20	0	60
asparagine (Asn)	N	1	4	2	2	0	6	4	2	2	0	6
aspartic acid (Asp)	D	2	4	3	1	0	5	8	6	2	0	10
cysteine (Cys)	C	0	3	1	1	1	5	0	0	0	0	0
glutamic acid (Glu)	E	3	5	3	1	0	7	15	9	3	0	21
glutamine (Gln)	Q	1	5	2	2	0	8	5	2	2	0	8
glycine (Gly)	G	2	2	1	1	0	3	4	2	2	0	6
histidine (His)	H	0	6	1	3	0	7	0	0	0	0	0
isoleucine (Ile)	I	3	6	1	1	0	11	18	3	3	0	33
leucine (Leu)	L	4	6	1	1	0	11	24	4	4	0	44
lysine (Lys)	K	6	6	1	2	0	12	36	6	12	0	72
methionine (Met)	M	0	5	1	1	1	9	0	0	0	0	0
phenylalanine (Phe)	F	4	9	1	1	0	9	36	4	4	0	36
proline (Pro)	P	1	5	1	1	0	7	5	1	1	0	7
serine (Ser)	S	2	3	2	1	0	5	6	4	2	0	10
tryptophan (Trp)	W	0	11	1	2	0	10	0	0	0	0	0
threonine (Thr)	T	1	4	2	1	0	7	4	2	1	0	7
tyrosine (Tyr)	Y	0	9	2	1	0	9	0	0	0	0	0
valine (Val)	V	2	5	1	1	0	9	10	2	2	0	18
chain ends			0	1	0	0	2	0	1	0	0	2
total		37	107	30	29	2	159	205	53	60	0	340

2) Theoretical carbon functions in LL-37

Amino acids	Carbon function in LL-37									
	CH ₃	CH ₂	CH	CCC	C-O	C-C-N	C-S	N-C=O, N-C-N, C=N	O=C-OH	Sum C
A	0	0	0	0	0	0	0	0	0	0
R	0	10	0	0	0	10	0	10	0	30
N	0	1	0	0	0	1	0	2	0	4
D	0	2	0	0	0	2	0	2	2	8
C	0	0	0	0	0	0	0	0	0	0
E	0	6	0	0	0	3	0	3	3	15
Q	0	2	0	0	0	1	0	2	0	5
G	0	0	0	0	0	2	0	2	0	4
H	0	0	0	0	0	0	0	0	0	0
I	6	3	3	0	0	3	0	3	0	18
L	8	4	4	0	0	4	0	4	0	24
K	0	18	0	0	0	12	0	6	0	36
M	0	0	0	0	0	0	0	0	0	0
F	0	4	20	4	0	4	0	4	0	36
P	0	2	0	0	0	2	0	1	0	5
S	0	0	0	0	2	2	0	2	0	6
W	0	0	0	0	0	0	0	0	0	0
T	1	0	0	0	1	1	0	1	0	4
Y	0	0	0	0	0	0	0	0	0	0
V	4	0	2	0	0	2	0	2	0	10
chain ends								-1	1	
sub total	19	52	29	4	3	49	0	44	5	205

3) Theoretical N functions in LL-37 and calculation of N1s peak distributions at pH 3.5

Amino acids	N fonction in LL-37		
	(CN)=O	C-N(H,C)	Sum N
A	0	0	0
R	5	15	20
N	2	0	2
D	2	0	2
C	0	0	0
E	3	0	3
Q	2	0	2
G	2	0	2
H	0	0	0
I	3	0	3
L	4	0	4
K	6	6	12
M	0	0	0
F	4	0	4
P	1	0	1
S	2	0	2
W	0	0	0
T	1	0	1
Y	0	0	0
V	2	0	2
chain ends	-1	1	0
sub total	38	22	60

Calculation of N function ratios in LL-37 at pH 3.5		
NH3+		
arginine (R): 1/3 of amin functions protonat		5
lysine (K):		6
end chain		1
total NH3+ at pH 3.5		12
NH2		
arginine (R): 2/3 of amin functions non protat		10
amide		
peptidic link + asp(N) + glut(Q)		38
Ratios		Peak
(amide + NH2)/Ntot	0.8	399.8 eV
NH3+/Ntot	0.2	401.7 eV

4) Calculation of protonation ratio CHI

		Total		Model HEP composition		Model CHI composition	
(Chi-Hep)2	Recorded peak	N1s		N1s		N1s	
	attribution	<u>N</u> H ⁺ -(C,H)	<u>N</u> -(C,H), C- <u>N</u> -SO ₃	<u>N</u> H ⁺ -(C,H)	<u>N</u> -(C,H), C- <u>N</u> -SO ₃	<u>N</u> H ⁺ -(C,H)	<u>N</u> -(C,H), C- <u>N</u> -SO ₃
	Binding energy	402	399.8	402	399.8	402	399.8
	Surface atomic fraction	1.2	1.7	0	0.5	1.2	1.2

5) HEP/LL-37 ratio for PPCs-coated samples by analysis of the N 1s peak (method 1)

(Chi-Hep)2/(PPCs/Chi)6	Model of HEP composition						Model of CHI composition					
	S	N	C	O	total	Unity* HEP	N	C	O	total	Unity* CHI	
(Chi-Hep)2/(PPCs/Chi)6	2.58	0.86	10.33	17.22	31.00	0.86	0.40	2.40	1.60	4.41	0.40	
(Chi-Hep)2/(PPCs/Chi)6 + MHB	1.96	0.65	7.83	13.05	23.48	0.65	0.00	0.00	0.00	0.00	0.00	
(Chi-Hep)2/(PPCs/Chi)6 + NaCl	2.15	0.72	8.58	14.31	25.75	0.72	0.00	0.00	0.00	0.00	0.00	
(Chi-Hep)2/(PPCs/Chi)6 + SDS	3.28	1.09	13.14	21.89	39.41	1.09	4.69	28.14	18.76	51.59	4.69	
	*unity = sodium HEP dimer (C ₁₄ H ₂₅ NO ₂₀ S ₃)						*unity = CHI monomer 95% deacetylated (C ₆ H ₁₁ NO ₄)					

(Chi-Hep)2/(PPCs/Chi)6	Model of LL-37 composition				
	N	C	O	total	unity* LL37
(Chi-Hep)2/(PPCs/Chi)6	9.58	32.74	8.31	50.63	0.16
(Chi-Hep)2/(PPCs/Chi)6 + MHB	10.17	34.75	8.81	53.74	0.17
(Chi-Hep)2/(PPCs/Chi)6 + NaCl	10.65	36.38	9.23	56.26	0.18
(Chi-Hep)2/(PPCs/Chi)6 + SDS	0.13	0.46	0.12	0.71	0.00
	*unity = one peptide				

The values in blue correspond to N_{LL-37} and N_{CHI} and were found by resolving the following equations explained in Section 1.5.5. of Results:

$$N^{399.8} = 0.8 N_{LL-37} + 0.5 N_{CHI} + N_{HEP}$$

$$N^{401.7} = 0.2 N_{LL-37} + 0.5 N_{CHI}$$

6) HEP/LL-37 ratio for PPCs-coated samples by analysis of the C 1s peak (2nd method)

C																				
C 1s																				
HEPARINE				CHITOSAN			LL37					SDS			AUTRE					TOTAL
O=C-O	O-C-O	C-(O,N)	C tot.	O-C-O	C-(O,N)	C tot.	O=C-O	N-C=O	C-(O,N)	C-(C,H)	C tot.	C-O	C-(C,H)	C tot.	O=C-O	C=O, O-C-O	C-(O,N)	C-(C,H)	C tot.	C tot.
															288.9	287.8	286.3	284.8		
															1.3	1.3	1.3	1.3		
															3.7	1.7	3.8	33.0	42.1	42.1
289.0	287.8	286.5		287.8	286.5										289.5	288.2	287.0	284.8		
1.4	1.4	1.4		1.4	1.4										1.4	1.4	1.4	1.4		
0.5	1.0	4.5	5.9	2.0	8.2	10.2									1.0	2.4	0.0	14.4	17.9	34.1
289.0	287.8	286.5		287.8	286.5		289.0	288.0	286.1	284.8					289.0		286.3	284.8		
1.4	1.4	1.4		1.4	1.4		1.4	1.4	1.4	1.4					1.4		1.4	1.4		
0.9	1.7	7.7	10.2	0.0	0.0	0.0	0.9	7.6	8.9	17.9	35.3				0.0		7.8	0.4	8.2	53.7
289.0	287.8	286.5		287.8	286.5		289.0	288.0	286.1	284.8					289.0		286.3	284.8		
1.4	1.4	1.4		1.4	1.4		1.4	1.4	1.4	1.4					1.4		1.4	1.4		
0.6	1.3	5.7	7.6	0.0	0.1	0.1	0.9	8.0	9.4	18.8	37.2				0.0		8.8	2.5	11.2	56.0
289.0	287.8	286.5		287.8	286.5		289.0	288.0	286.1	284.8					289.0		286.3	284.8		
1.4	1.4	1.4		1.4	1.4		1.4	1.4	1.4	1.4					1.4		1.4	1.4		
0.7	1.4	6.4	8.5	0.1	0.4	0.5	0.9	8.1	9.6	19.2	37.9				0.0		7.4	1.5	8.9	55.8
289.0	287.8	286.5		287.8	286.5		289.0	288.0	286.1	284.8		286.3	284.8		289.0		286.7	284.8		
1.3	1.3	1.3		1.3	1.3		1.3	1.3	1.3	1.3		1.3	1.3		1.3		1.3	1.3		
0.2	0.4	2.0	2.7	0.0	0.0	0.0	0.5	4.1	4.9	9.8	19.3	2.4	26.3	28.7	0.9		10.6	0.8	12.3	63.0

	BALANCE S				BALANCE N					
	S-HEPARINE	S-SDS	S DETECTED	S deduced/S detected	N-HEPARINE	N-CHITOSAN	N-LL37	N-DETECTE	N deduced/N detected	
Ti Wafer	0.0	0.0	0.0		0.0	0.0	0.0	0.9		
(Chi-Hep)2	1.5	0.0	1.4	0.95	0.5	1.7	0.0	2.9	1.34	presence of
(Chi-Hep)2/(PPCs/Chi)6	2.6	0.0	2.6	1.00	0.9	0.0	10.3	10.8	0.96	
(Chi-Hep)2/(PPCs/Chi)6 MHB	1.9	0.0	1.9	1.03	0.6	0.0	10.9	10.8	0.94	
(Chi-Hep)2/(PPCs/Chi)6 NaCl	2.1	0.0	2.1	0.99	0.7	0.1	11.1	11.2	0.95	
(Chi-Hep)2/(PPCs/Chi)6 SDS	0.7	2.4	3.3	1.07	0.2	0.0	5.6	5.9	1.01	
				error<5%					error<5%	