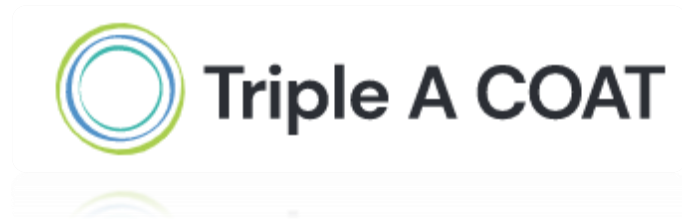


The Triple-A-COAT project

Fighting microbes the natural way



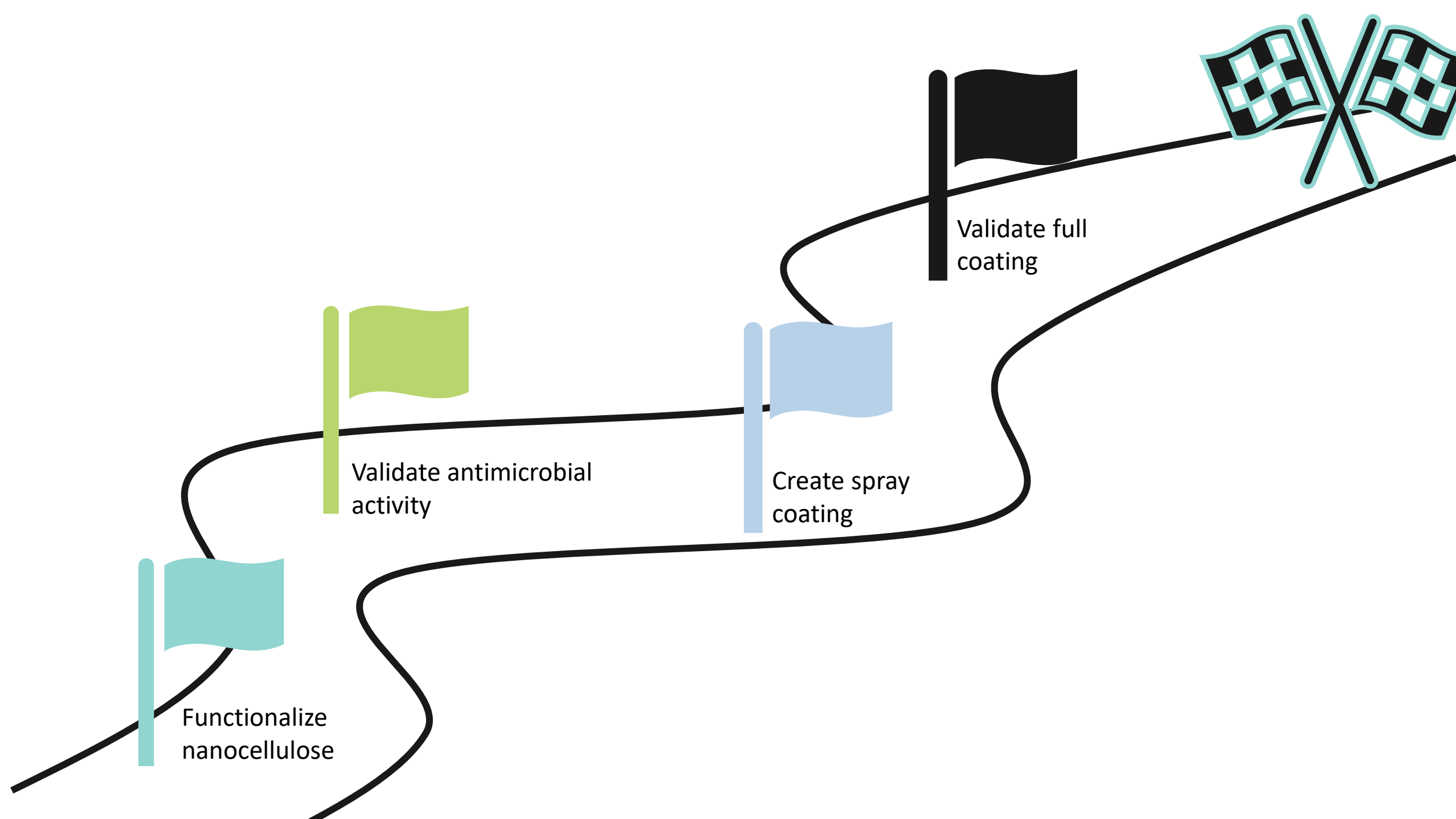
Antimicrobial, Antifungal & Antiviral Nanocoatings

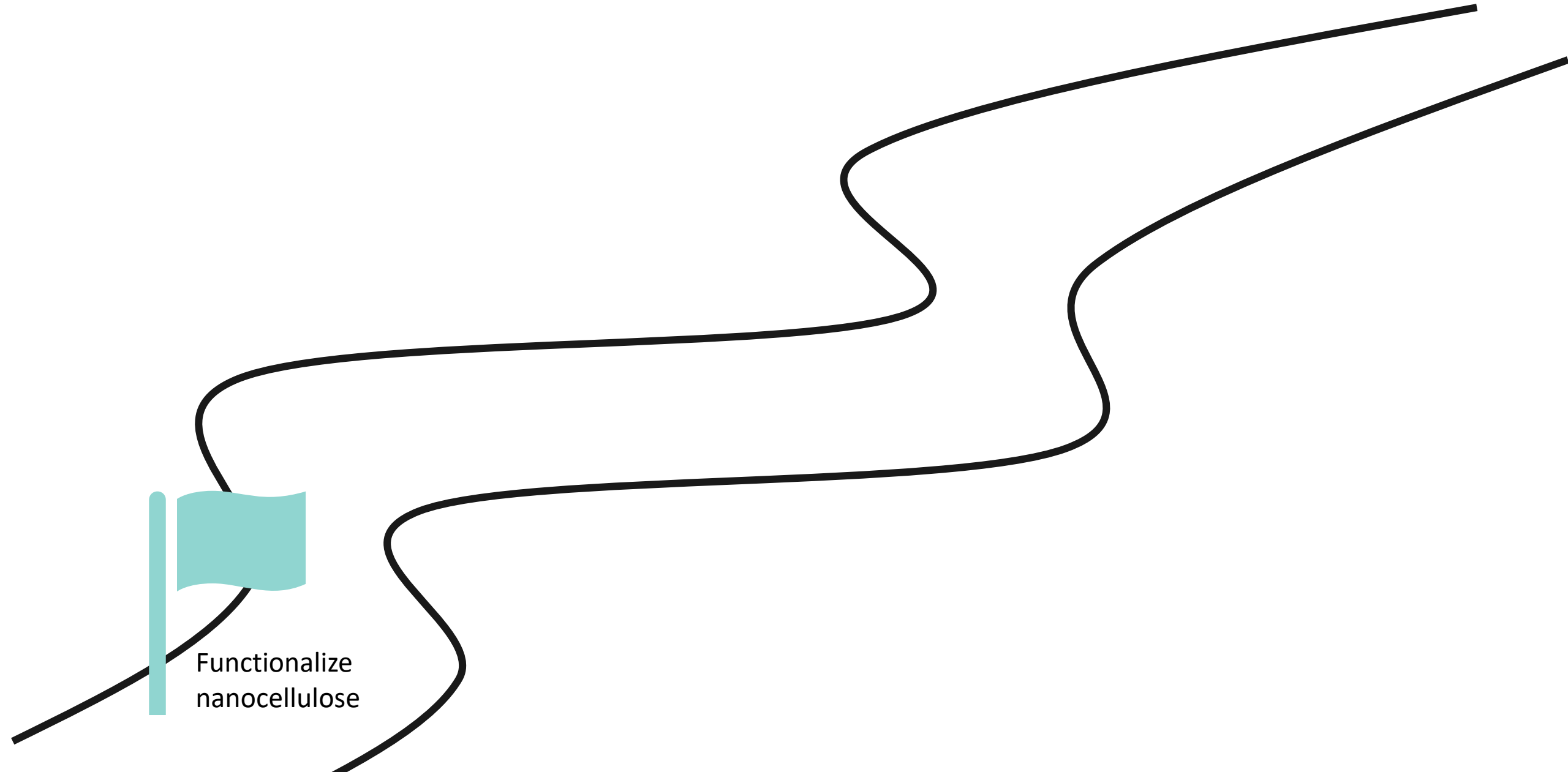
[Learn more >](#)



Ultimate Goal: Antimicrobial Coatings

- Sustainable
- Biobased
- Evolutionary proof
- Highly effective

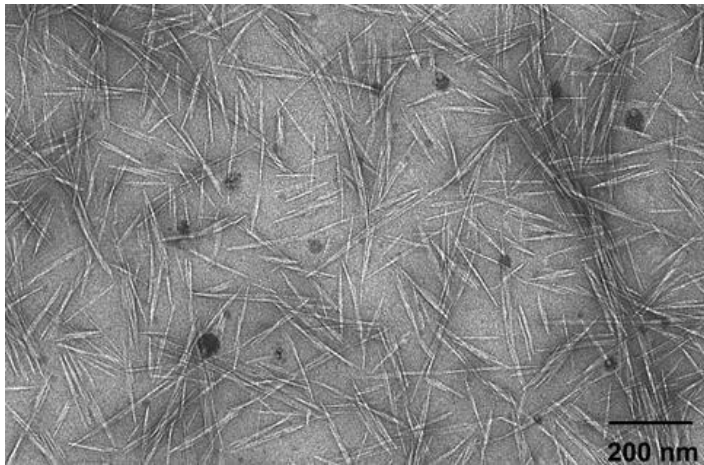




Functionalize
nanocellulose



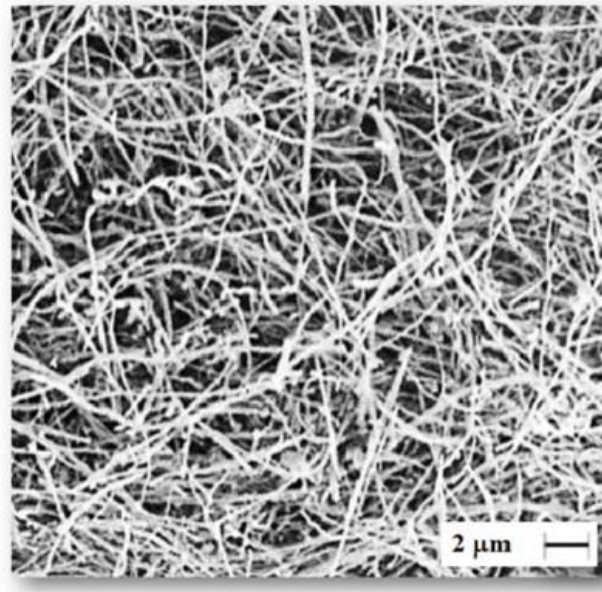
Cellulose nanocrystals (CNC)



CNC



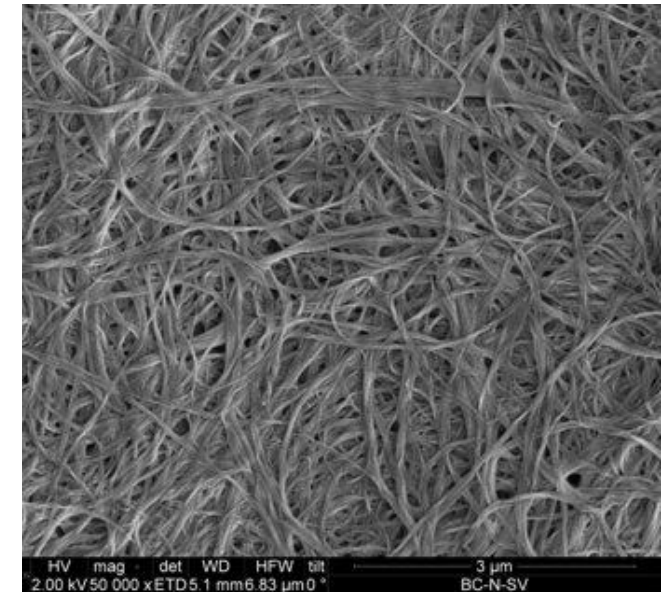
Cellulose nanofibrils (CNF)



CNF



Bacterial nanocellulose (BNC)



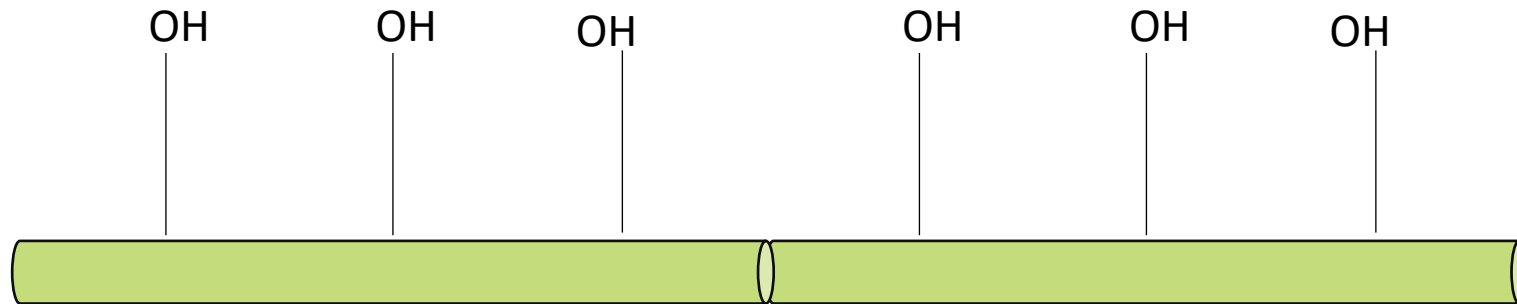
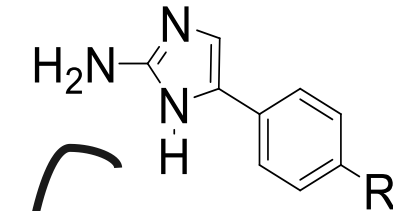
BNC

2-aminoimidazoles

Ion-ion interactions

Amination

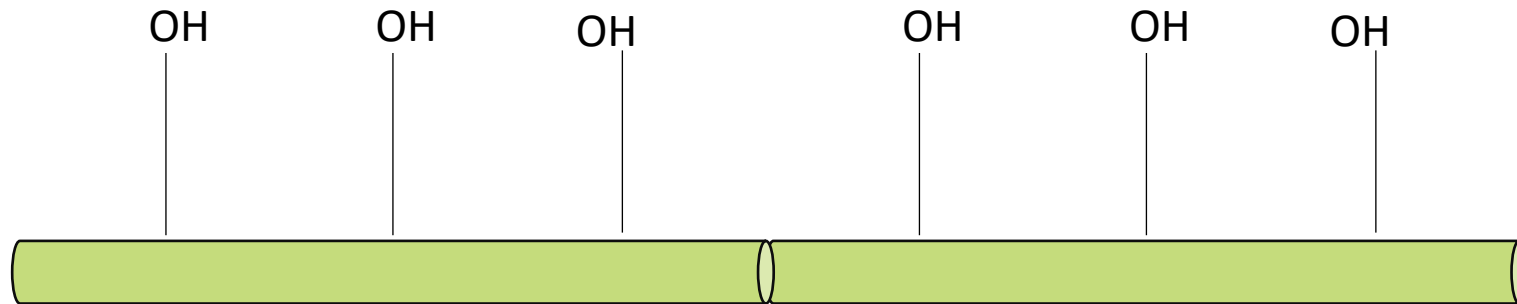
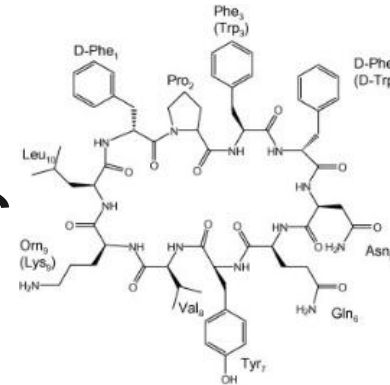
CNC, CNF & BNC



Antimicrobial peptides

Non-covalent bonding

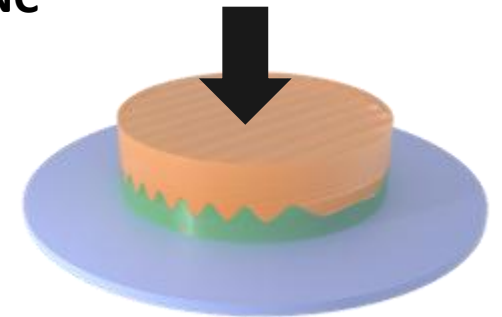
CNC, CNF & BNC



Nanopatterns

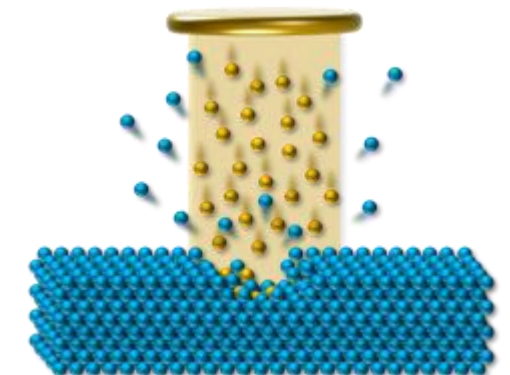
WET-NIL

CNC



Ion milling

CNC, CNF & BNC

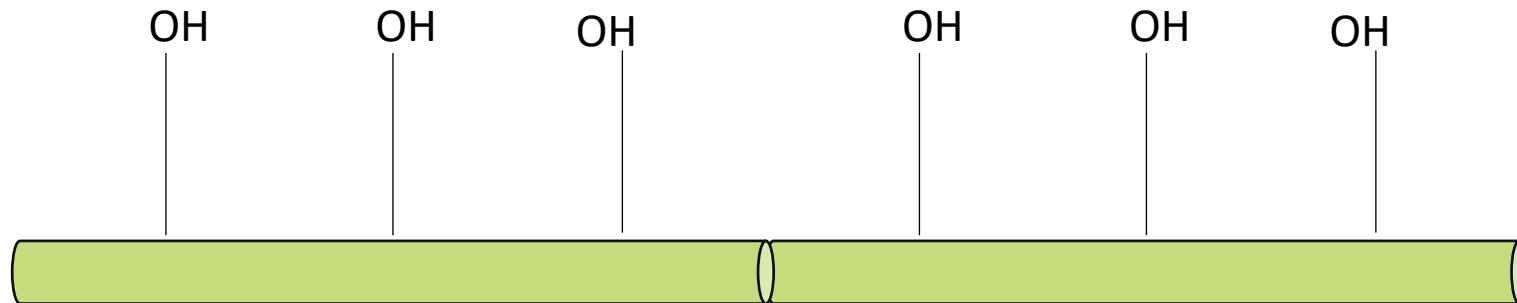
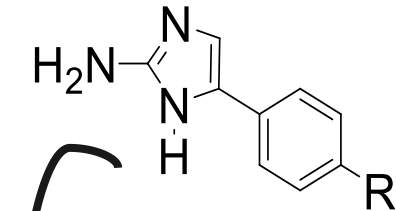


2-aminoimidazoles

Ion-ion interactions

Amination

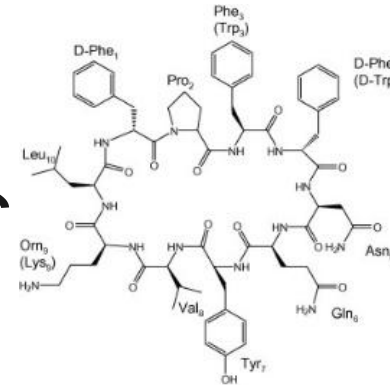
CNC, CNF & BNC



Antimicrobial peptides

Non-covalent bonding

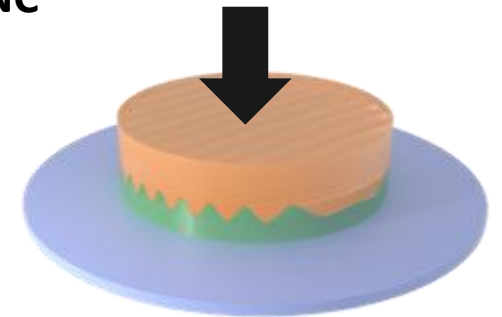
CNC, CNF & BNC



Nanopatterns

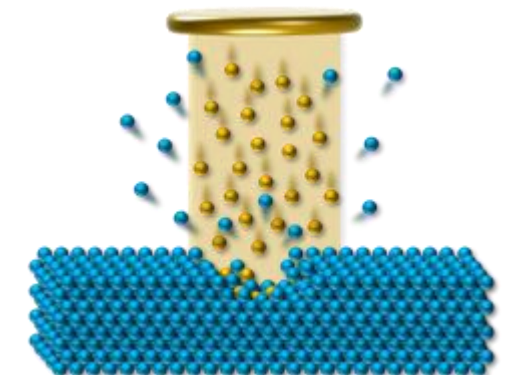
WET-NIL

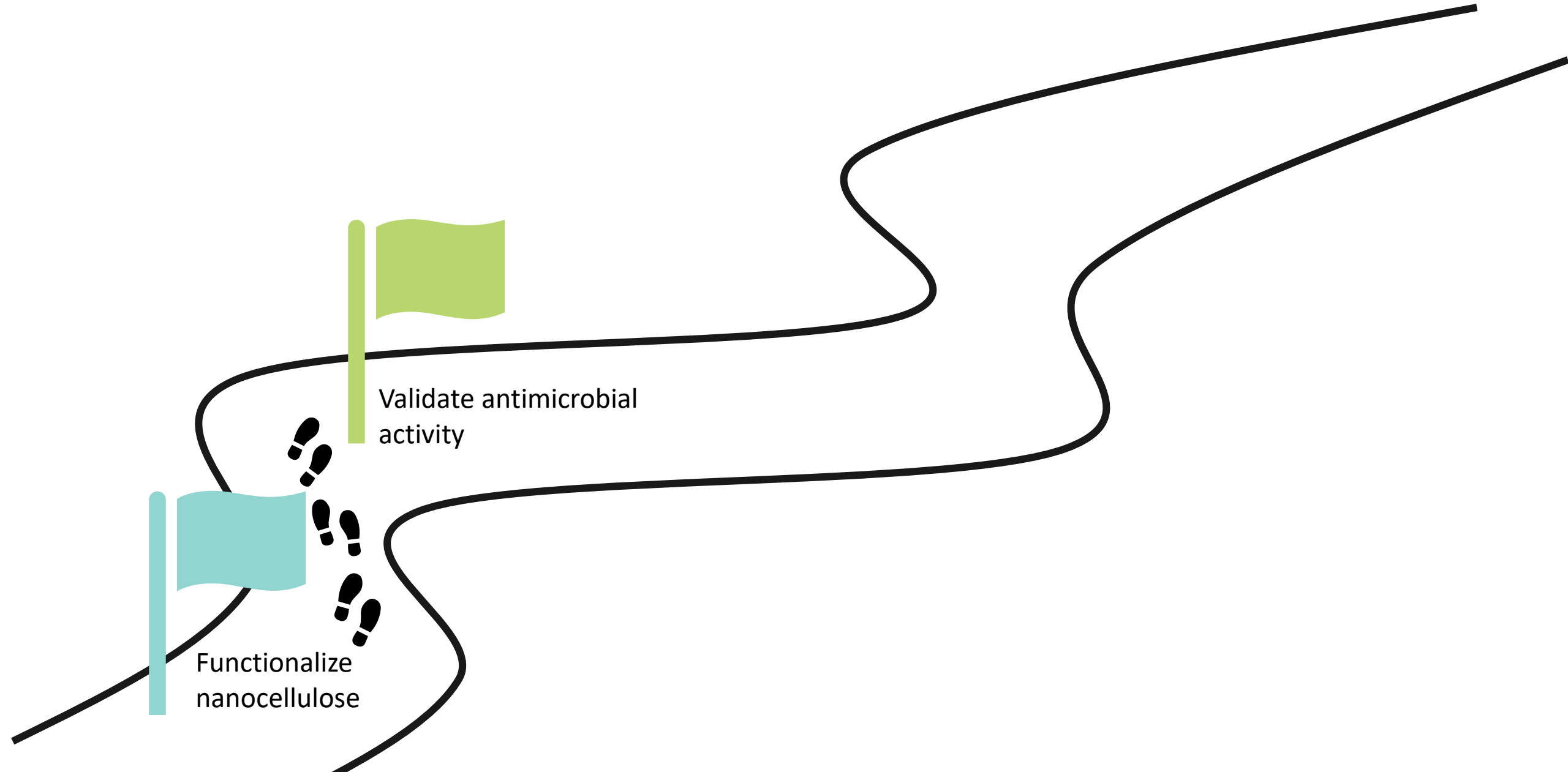
CNC



Ion milling

CNC, CNF & BNC

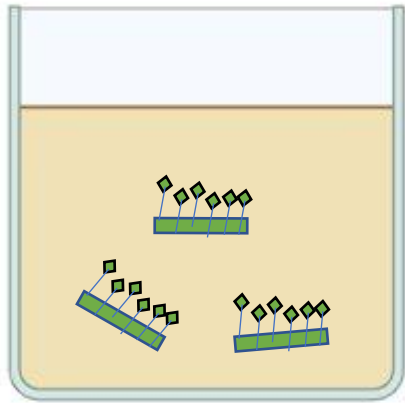




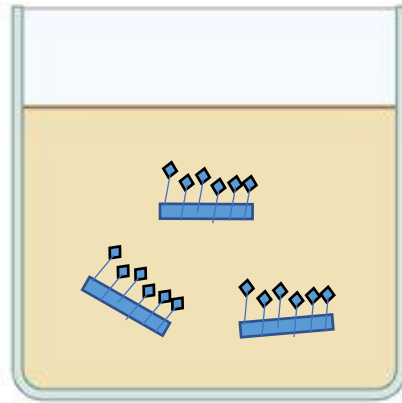
Functionalize
nanocellulose

Validate antimicrobial
activity

In suspension

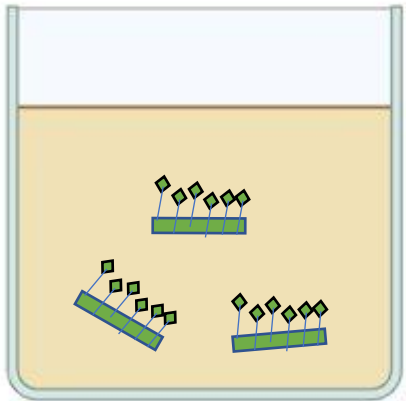


CNF+2AI
CNC+2AI
BNC+2AI



CNF+AMP
CNC+AMP
BNC+AMP

In suspension



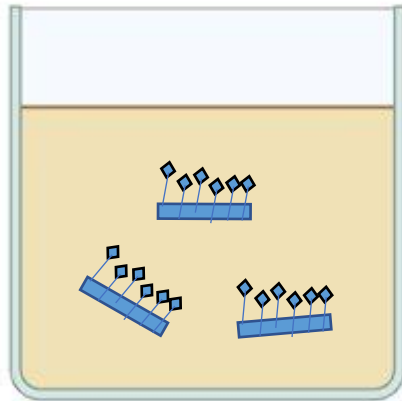
CNF+2AI

CNC+2AI

BNC+2AI

✓ Bacteria

✓ Fungi



CNF+AMP

CNC+AMP

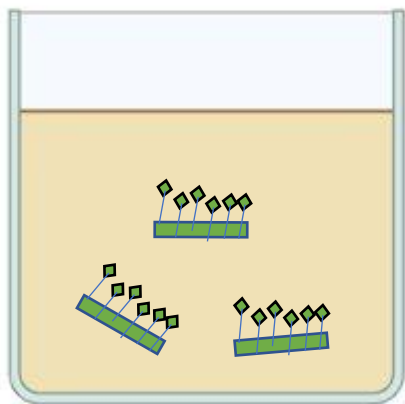
BNC+AMP

✓ Bacteria

✓ Fungi

✓ Viruses

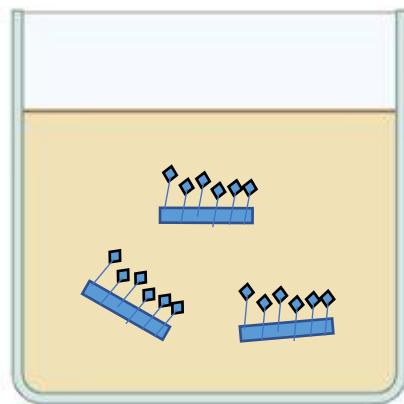
In suspension



CNF+2AI

CNC+2AI

BNC+2AI



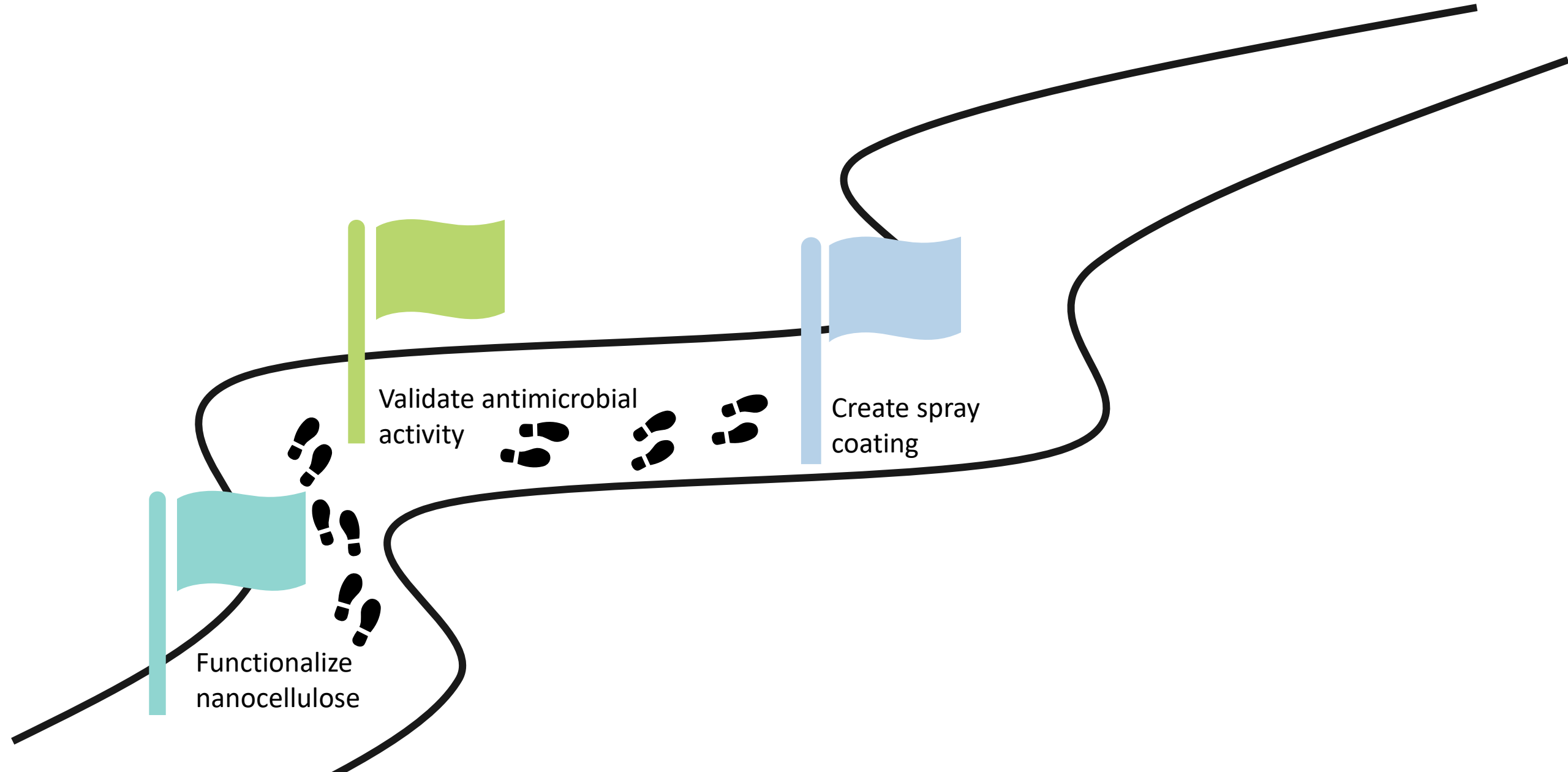
CNF+AMP

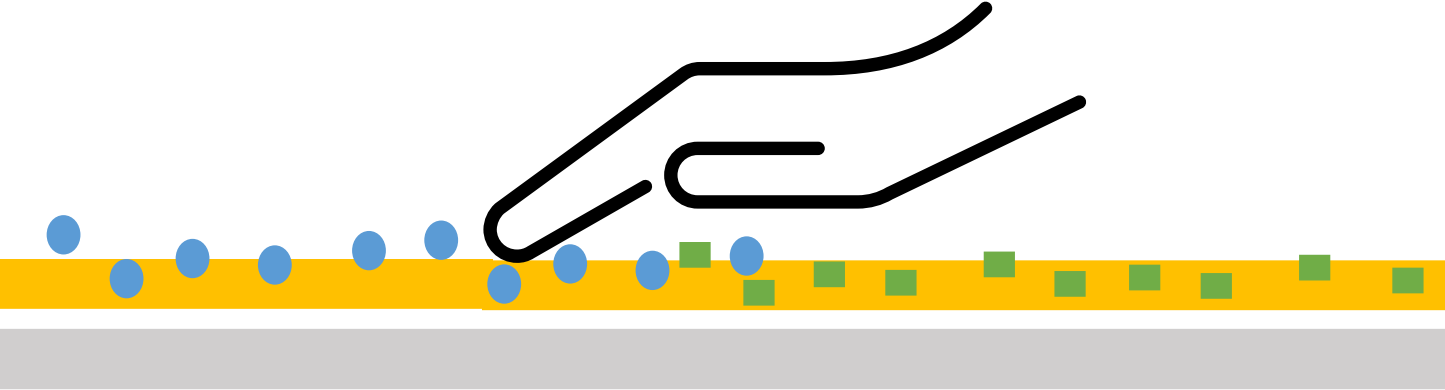
CNC+AMP

BNC+AMP

On a substrate







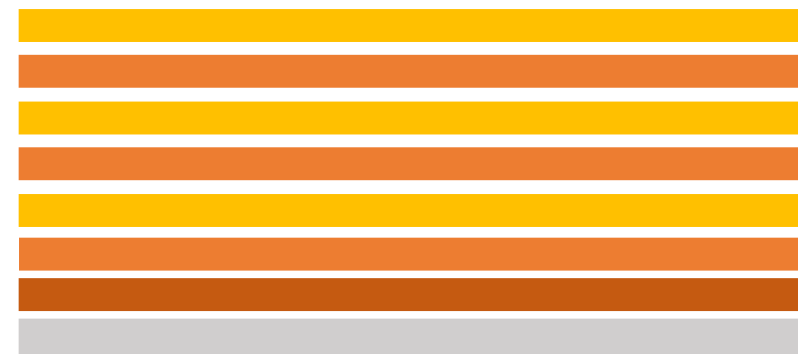
AQiBond™ system

CNC
AQiBond™



✓ CNC

PDA+BA system



CNF
BA
PDA

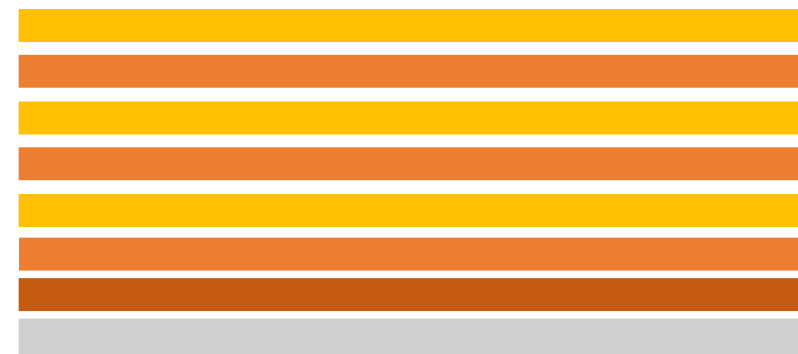


✓ CNF

AQiBond™ system

PDA+BA system

CNC
AQiBond™



CNF
BA
PDA

10 alternating layers



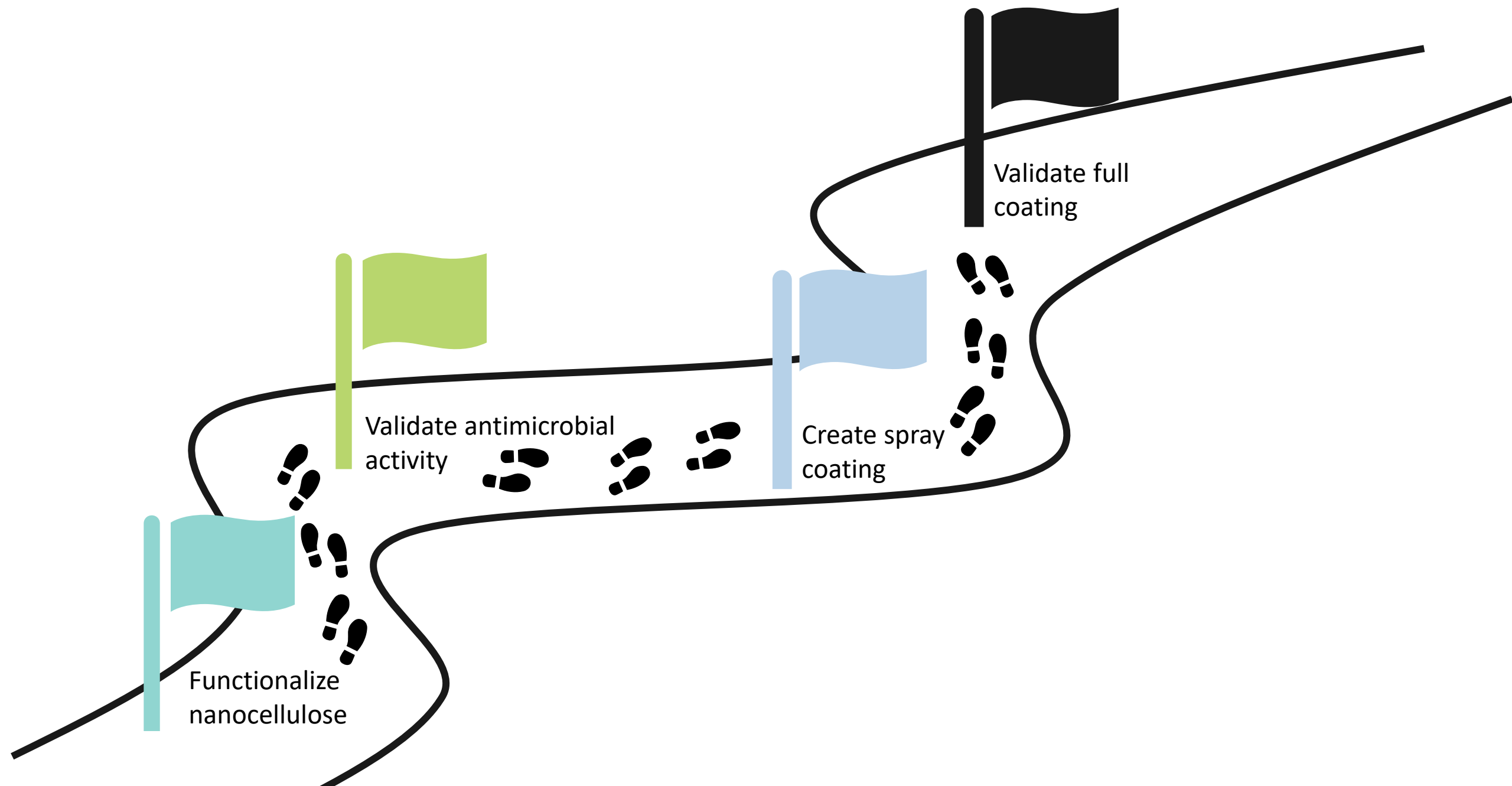
CNC

AMP

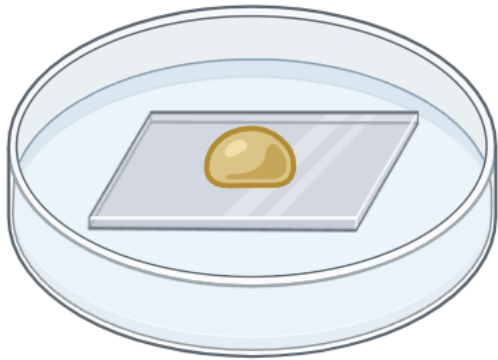


CNF

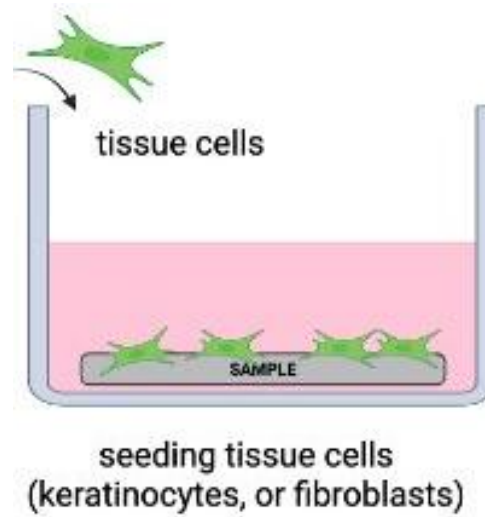
2-AI



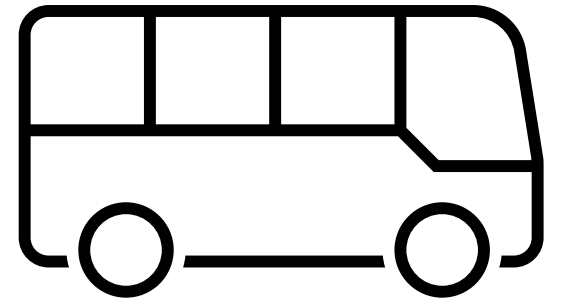
Antimicrobial activity



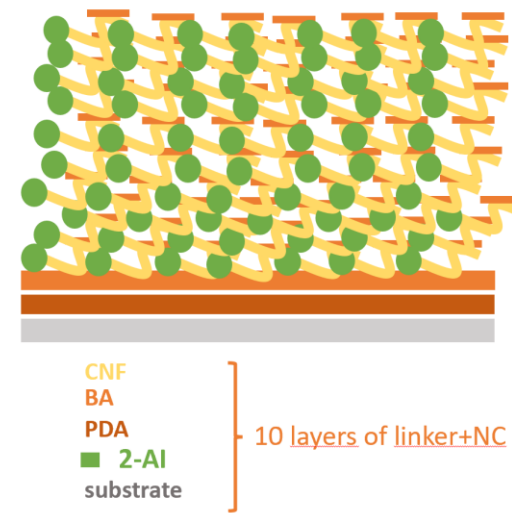
Toxicity



Functionality



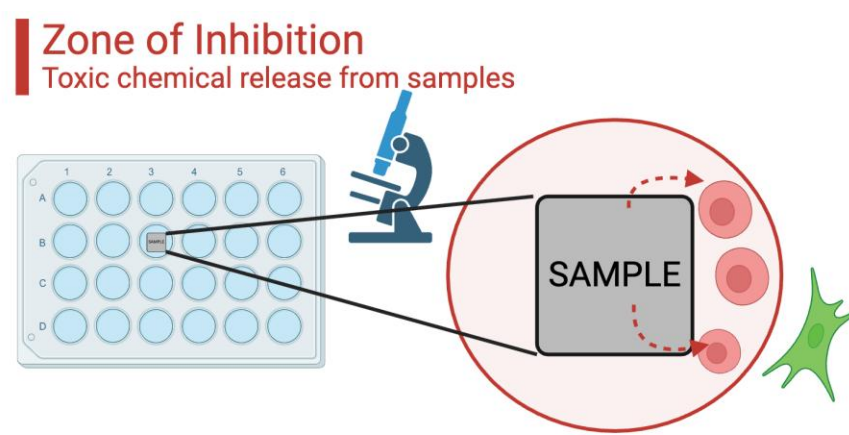
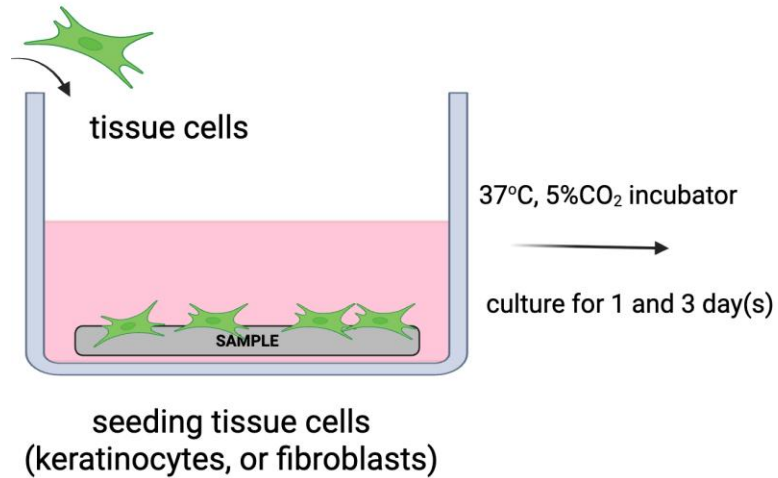
Antimicrobial activity



	AMP		2-AI	
	100 ug/mL	50 ug/mL	0.3 mmol/g	0.15 mmol/g
 <i>E. coli</i>				
 <i>S. aureus</i>				
 <i>C. albicans</i>				
<i>Human coronavirus</i>			Being tested	

Toxicity

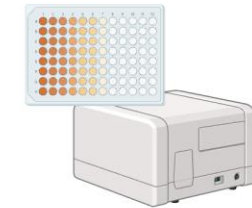
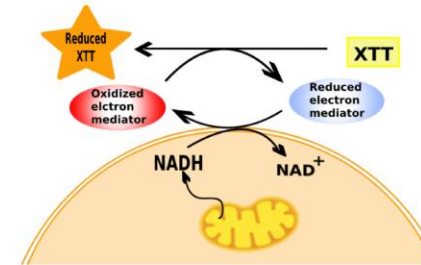
Toxicity Tests



XTT Assay

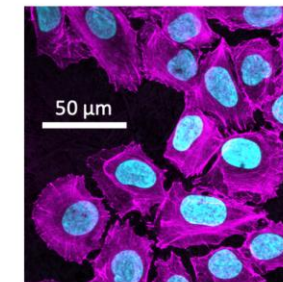
Cytotoxicity evaluation

Colorimetric reaction to determine cell viability



Confocal microscopy

Cell morphology



staining, imaging & analysis (cell adhesion, spreading, and morphology)

- “Zone of Inhibition” – released chemicals
- XTT assay – cell viability
- Fluorescent images – cell morphology (adhesion & spreading)

AMPs

Assays	Keratinocytes				Fibroblasts			
	CNC	CNC+ AQiBond™	CNC+ AQiBond™+ CDP-T3:G (50µg/mL)	CNC+ AQiBond™+ CDP-T3:G (100µg/mL)	CNC	CNC+ AQiBond™	CNC+ AQiBond™+ CDP-T3:G (50µg/mL)	CNC+ AQiBond™+ CDP-T3:G (100µg/mL)
Direct contact								
Fluorescent Images	●	●	●	●	●	●	●	●
XTT assay	●	●	●	●	●	●	●	●
Zone of Inhibition	●	●	●	●	●	●	●	●
Extraction								
XTT assay	●	●	●	●	●	●	●	●

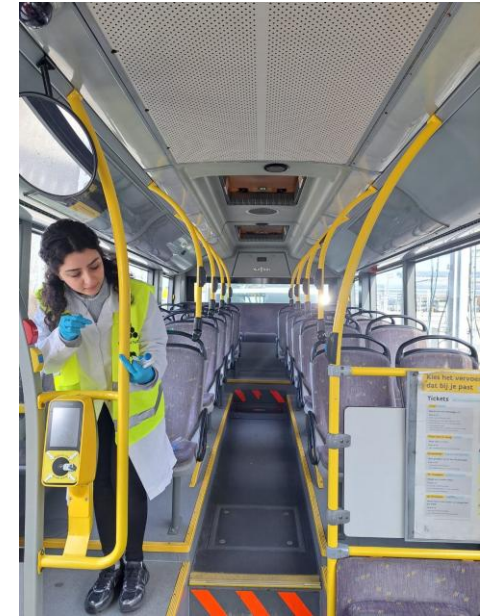
Nanopatterns

Assays	NIL-CNC	
	keratinocytes	fibroblasts
Fluorescent Images		
XTT assay		
Zone of Inhibition		

Functionality



Swabbing Surfaces on a De Lijn Bus



Bacterial Consortium

Strain(rep)	%	References
Staphylococcus pasteurii (CoNS)	30%	Ethiopia study: CoNS was 40.3% and <i>S.aureus</i> 27.5% https://doi.org/10.3389/fpubh.2025.1521479
Bacillus haynesii (Bacillus genus)	25%	<i>Bacillus</i> spp. (Gram-positive bacilli) made up ~25.2% of isolates in one culture study https://doi.org/10.3389/fpubh.2025.1521479
Pseudomonas luteola (Pseudomonas genus)	20%	Harar Ethiopia found Pseudomonas ~8.9% https://doi.org/10.3389/fpubh.2025.1521479
Acinetobacter pecorum (Acinetobacter genus)	15%	Mexico City subway surfaces had ~10.5% <i>Acinetobacter</i> (the highest genus) https://doi.org/10.1371/journal.pone.0237272
Pantoea conspicua	10%	<i>Pantoea</i> is rarely reported, but did appear in one transit survey https://doi.org/10.1038/s41598-024-56225-9

Sampling Plan

Contaminate and Swab Once per Week

JUNE - JULY

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
31	1	2	3	4	5	6
7	8	9	10	11  2h swab	12 24h swab	13
14	15	16	17	18  24h swab	19	20
21	22	23	24	25  24h swab	26	27
28	29	30	1	2	3	4

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
28	29	30	1	2  24h swab	3	4
5	6	7	8	9  2h swab	10 24h swab	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
26	27	28	29	30	31	1



Spray contaminate
the bus

2h swab

24h swab

Swab after 2 h

Swab after 24 h

Sampling Plan

Contaminate and Swab Once per Week

JUNE - JULY

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
31	1	2	3	4	5	6
7	8	9	10	11  2h swab	12 24h swab	13
14	15	16	17	18  24h swab	19	20
21	22	23	24	25  24h swab	26	27
28	29	30	1	2	3	4

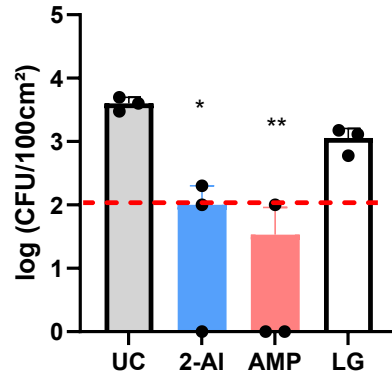
SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
28	29	30	1	2  24h swab	3	4
5	6	7	8	9  2h swab	10 24h swab	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
26	27	28	29	30	31	1

Higher inoculum
during 5th week!

Video link

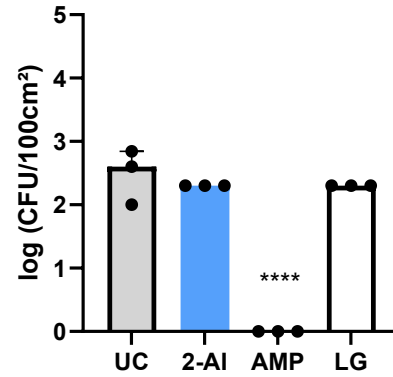
WEEK 1

Consortium Recovery on Textile after 1st infection (24h)



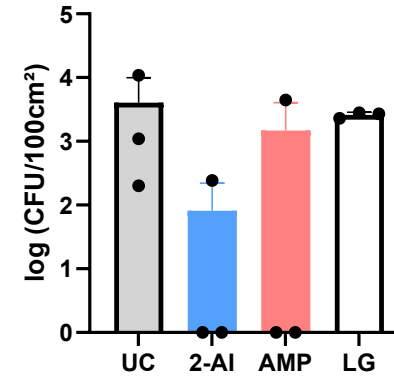
WEEK 2

Consortium Recovery on Textile after 2nd infection (24h)



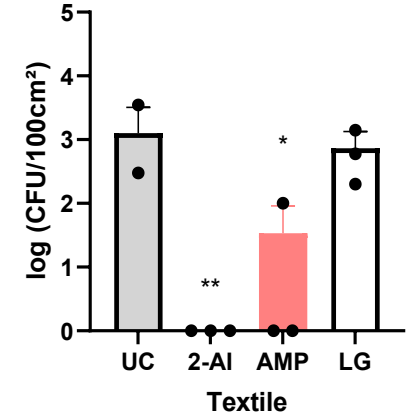
WEEK 3

Consortium Recovery on Textile after 3rd infection (24h)

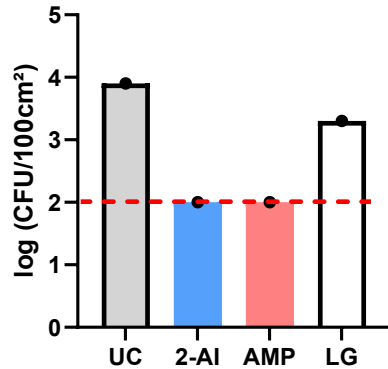


WEEK 4

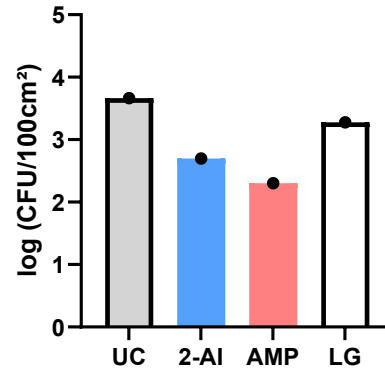
Consortium Recovery on Textile after 4th infection (24h)



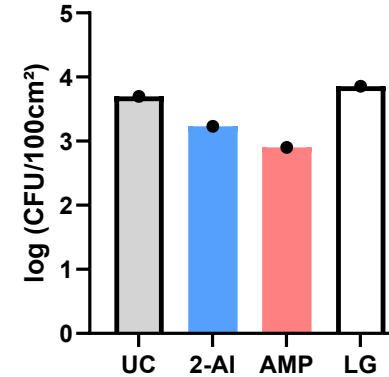
Consortium Recovery on poles after 1st infection (24h)



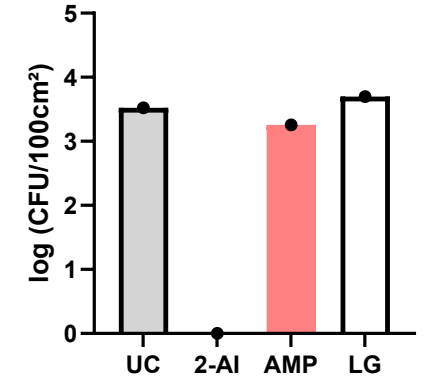
Consortium Recovery on poles after 2nd infection (24h)



Consortium Recovery on poles after 3rd infection (24h)



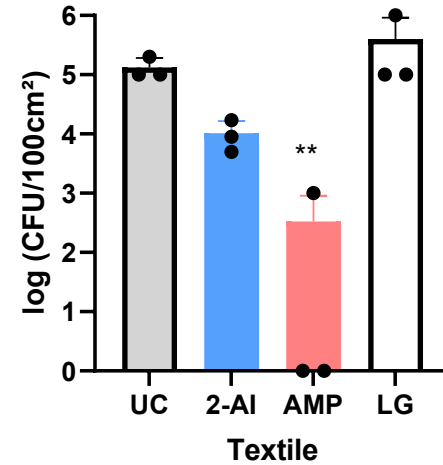
Consortium Recovery on poles after 4th infection (24h)



WEEK 5



Consortium Recovery on Textile
after 5th infection (24h)

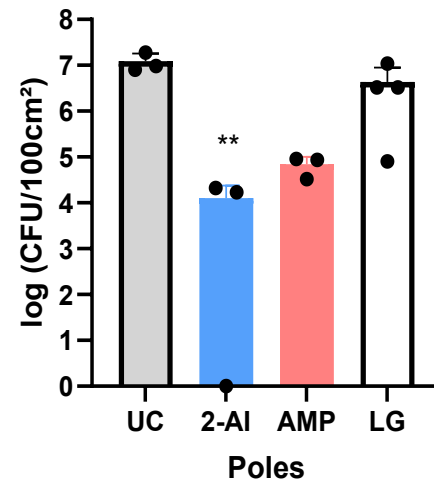


Seats/5th infection: AMP lowers CFU after 24 hours

Higher inoculum!



Consortium Recovery on poles
after 5th infection (24h)



Poles/5th infection: 2-AI lowers after 2 & 24 hours

Acknowledgements



Acknowledgements

European Union

Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or The European Health and Digital Executive Agency. Neither the European Union nor the granting authority can be held responsible for them

