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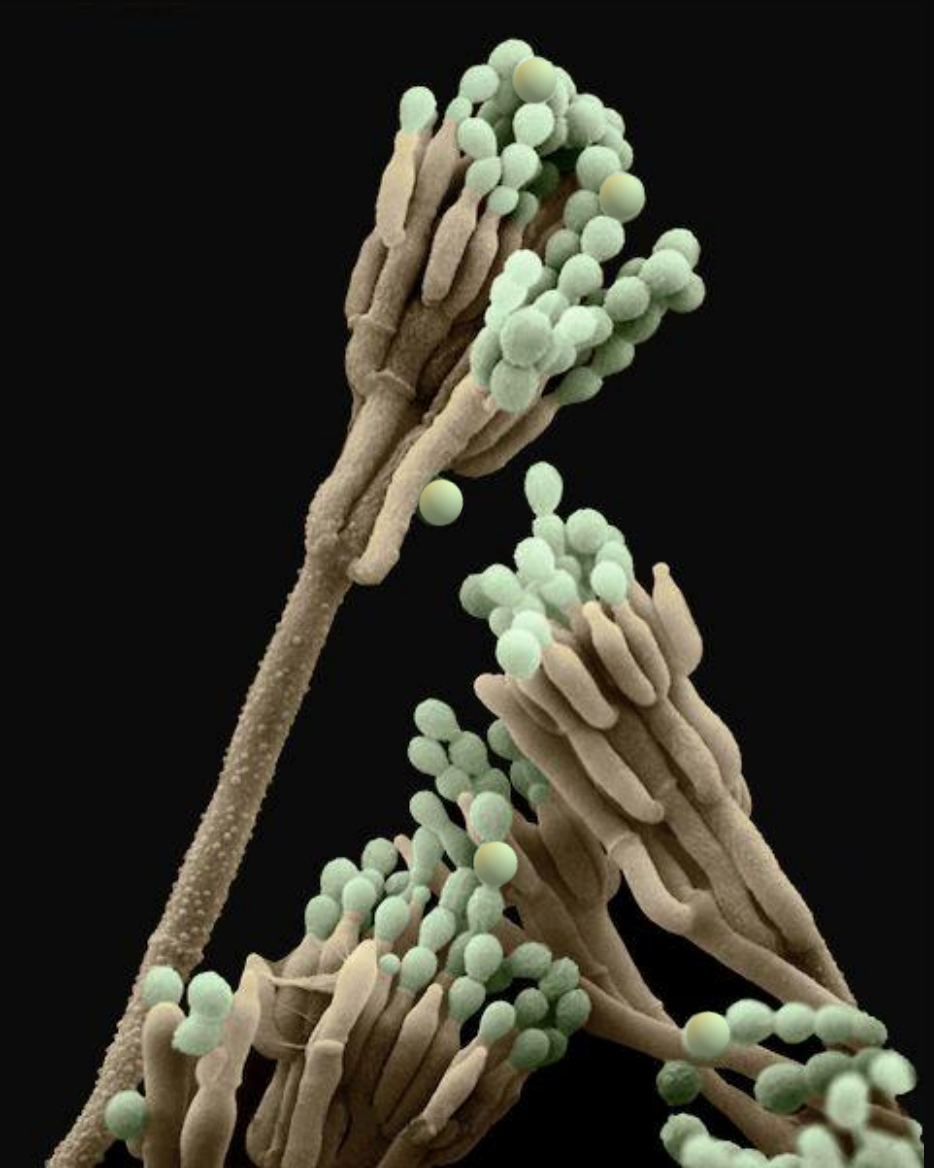
Ministero dell'Università e della Ricerca

Italiadomani
PIANO NAZIONALE DI RIPRESA E RESILIENZA

DEVELOPMENT OF NEW BIOLOGICAL DRUGS FOR THE TREATMENT OF FUNGAL INFECTIONS

Università degli Studi di Urbino
23 maggio 2024

ALARMING NUMBERS



13 million people affected by fungal infections

1.5 million deaths every year

>90% caused by Candida spp., Cryptococcus spp. Aspergillus spp.

Candidemia alone represents **60%** of total fungal infections

According to CDC, ***C. auris*** is the **1st** most urgent threats

Just **3** major classes of antifungal drugs

>90% are resistant to **azoles**
>30% are resistant to **2** antifungal classes
3-5% to **all** antifungals

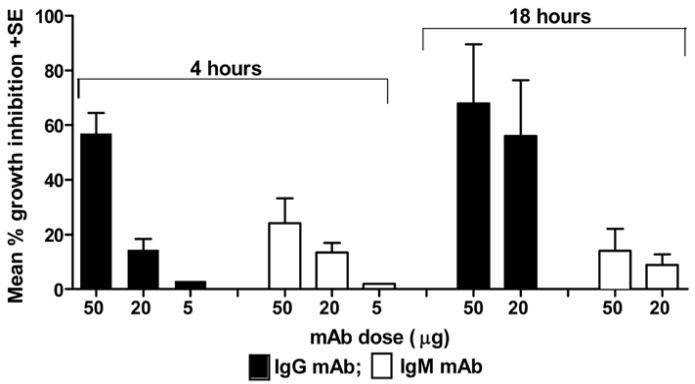
www.cdc.gov
www.gaffi.org

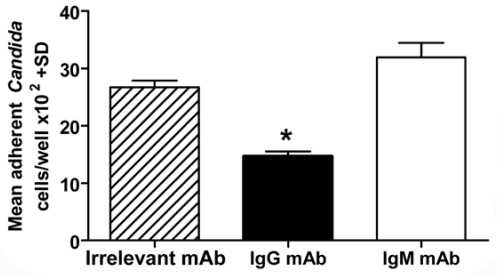
THE BIRTH OF ANTIBODIES
ANTI-β-1,3-GLUCANS

Torosantucci et al. 2009

IgG_{2b} 2G8

IgM 1E12



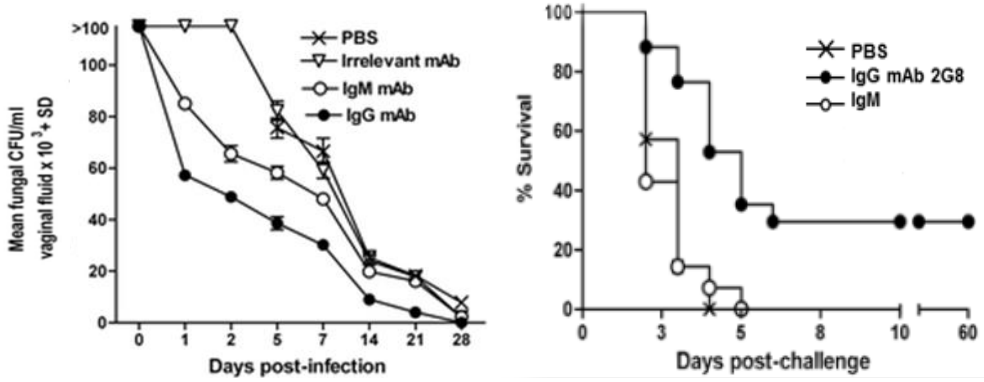


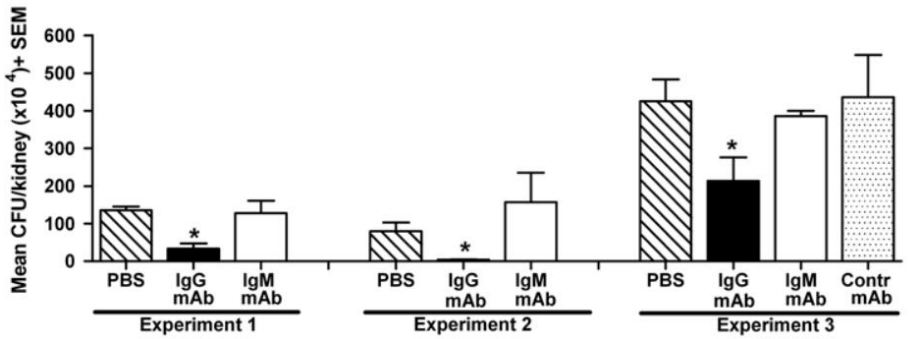
In vitro

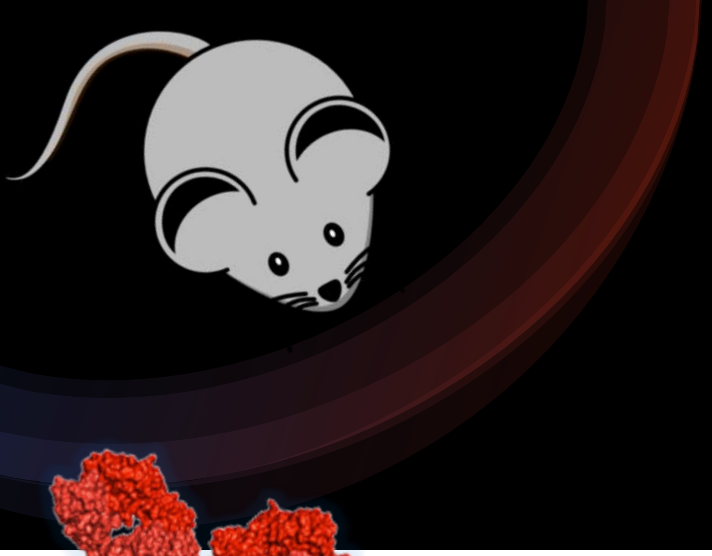
IgG_{2b} 2G8

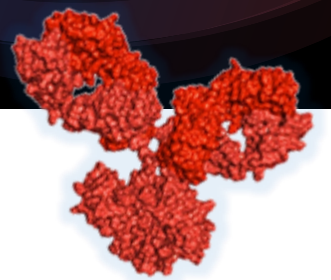
- is specific for β-1,3-Glucans
- performed always better than IgM 1E12
- conferred protection against mucosal and systemic candidiasis

In vivo

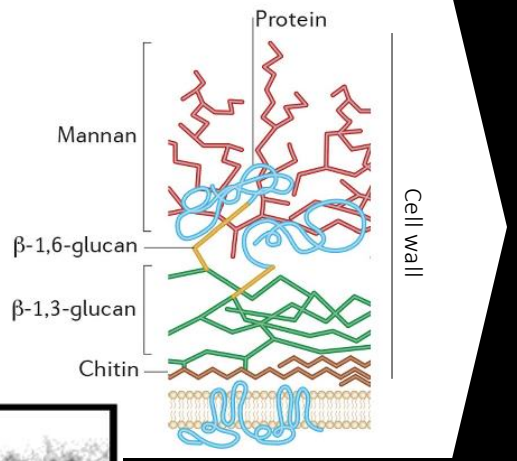


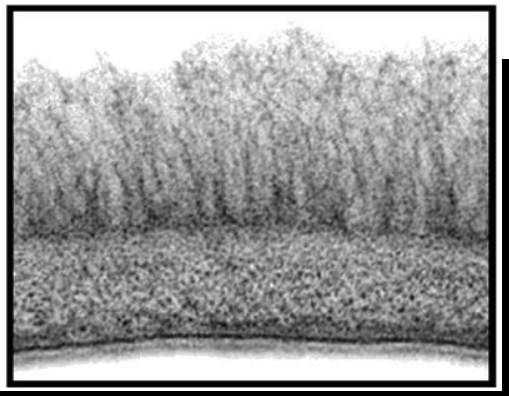






IgG_b 2G8

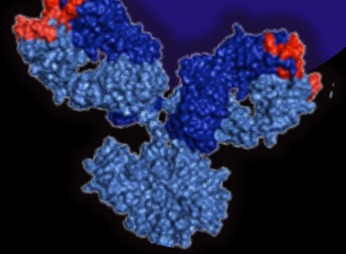




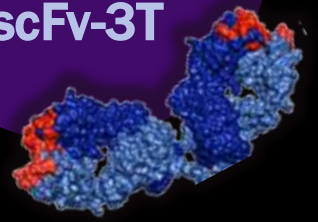
C. albicans

N. Gow et al. 2017

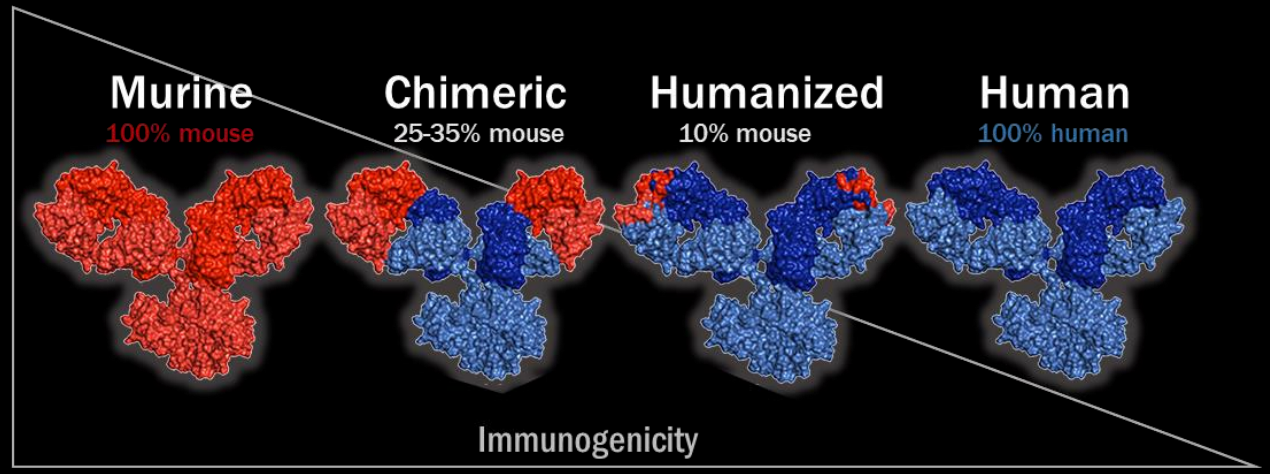
Humanization processes



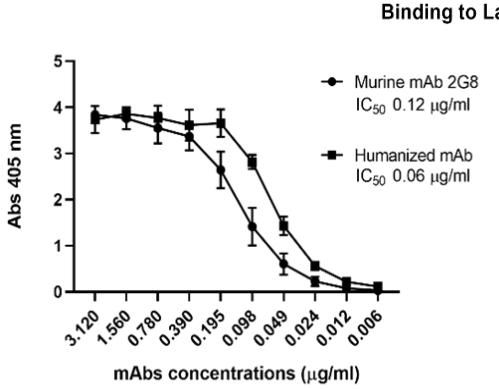
Full-length antibody
Dia-T51

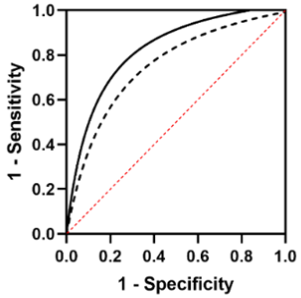


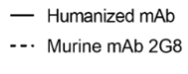
Single-chain fragment variable
scFv-3T

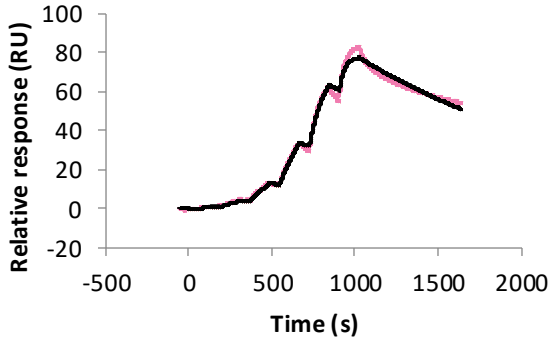


Dia-T51 binding *in vitro*



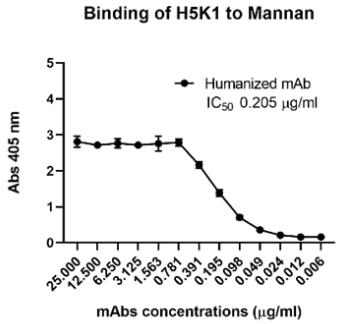


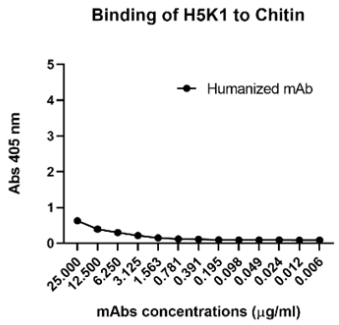


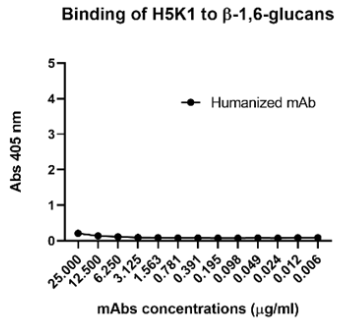


Murine-humanized mAb 2G8 comparison

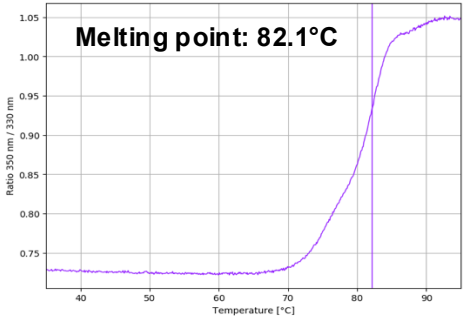
	IC_{50} (µg/ml)	AUC	K_D
Dia-T51	0.06	0.85	$3.59x10^{-9}$
2G8	0.12	0.77	$1.9x10^{-9}$



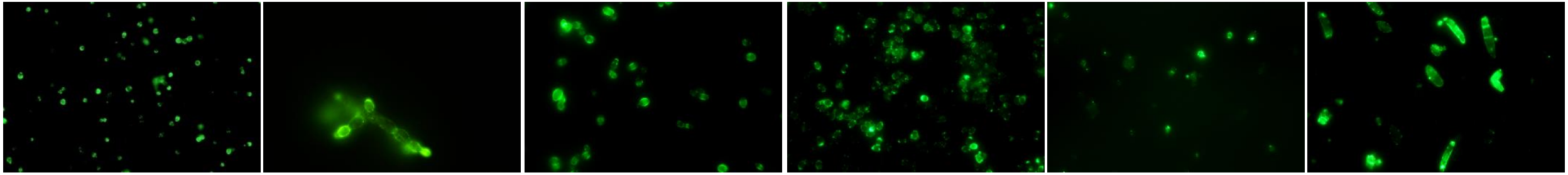




Stability



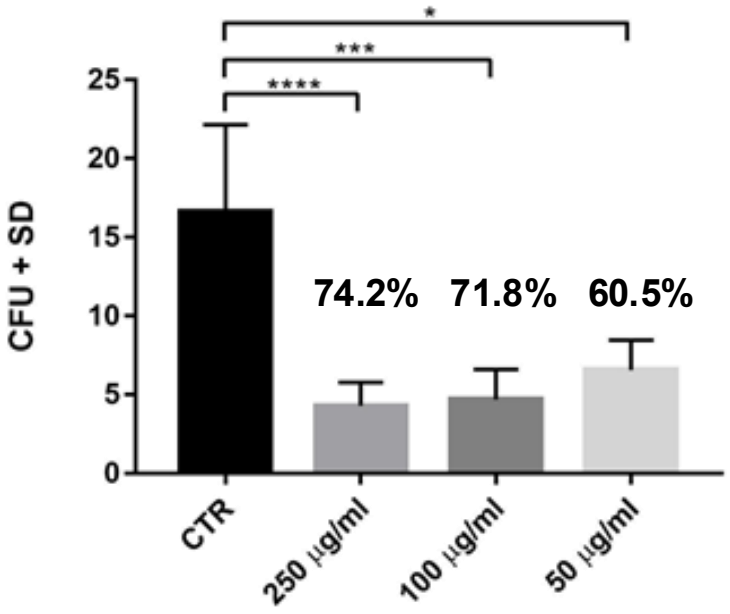
Dia-T51 IC_{50} (µg/ml)	6 months 4°C	12 months 4°C	18 months -80°C
	0.10 (K_D $3.37x10^{-9}$)	0.10	0.09



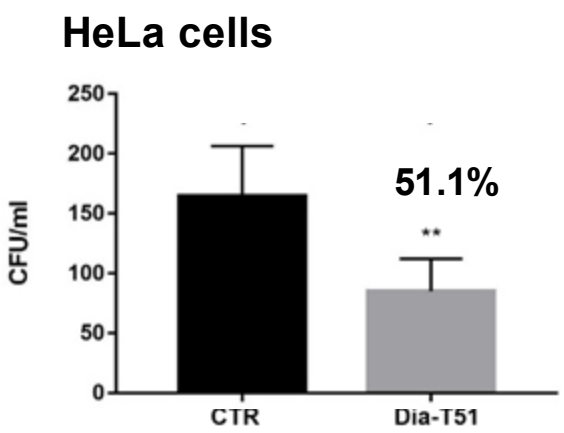
C. auris | *C. albicans* hyphal form | *C. albicans* yeast form | *C. glabrata* | *A. fumigatus* conidia | *F. solani* conidia

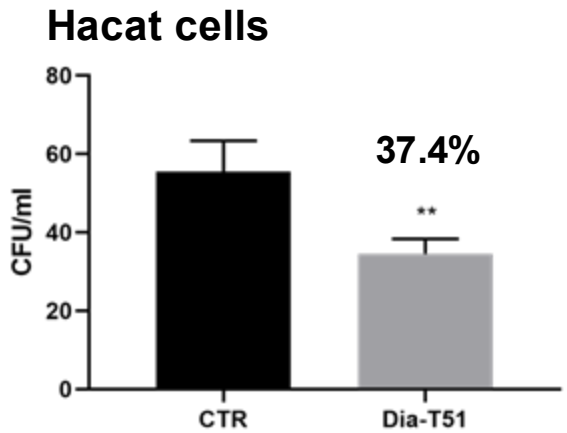
Dia-T51 effect alone - *C. auris*

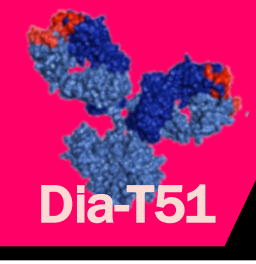
C. auris growth inhibition



C. auris adhesion inhibition

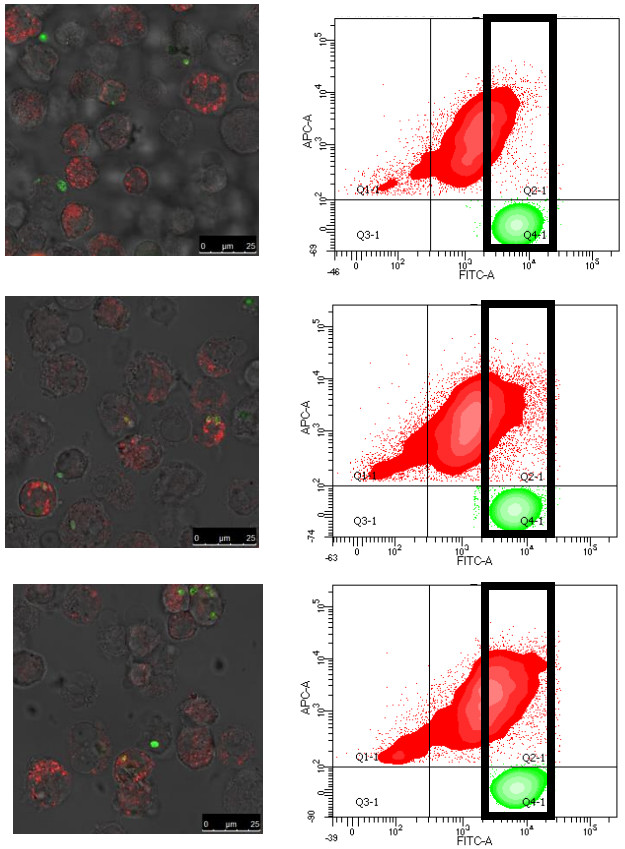






Dia-T51 effect alone - *C. auris*

THP-1 cell line

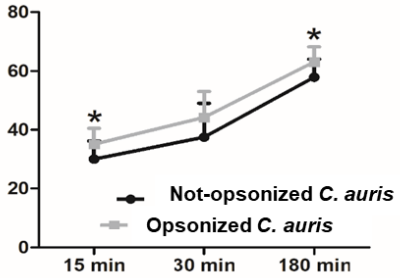


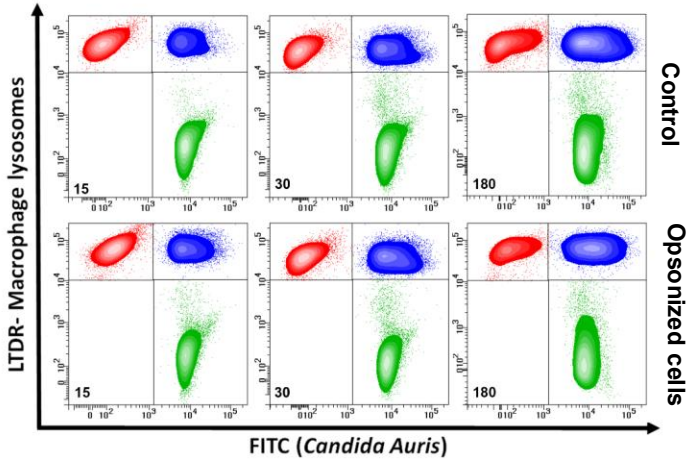
Red population: THP-1 (human macrophages)
Green population: *C. auris*

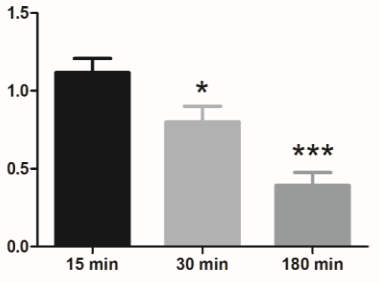
	Engulfing macrophages (%)	Residual Candida/Macrophages	Phagocytosis rate (%)
Not-opsonized *C. auris*	84.5	3.79	37.0
Opsonized *C. auris (2.5 µg/ml Dia-T51)*	85.8	2.44	46.5
Opsonized *C. auris (25 µg/ml Dia-T51)*	87.0	2.33	65.6

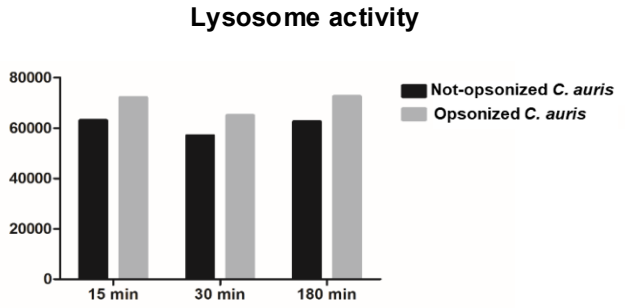
Human monocytes-derived macrophages

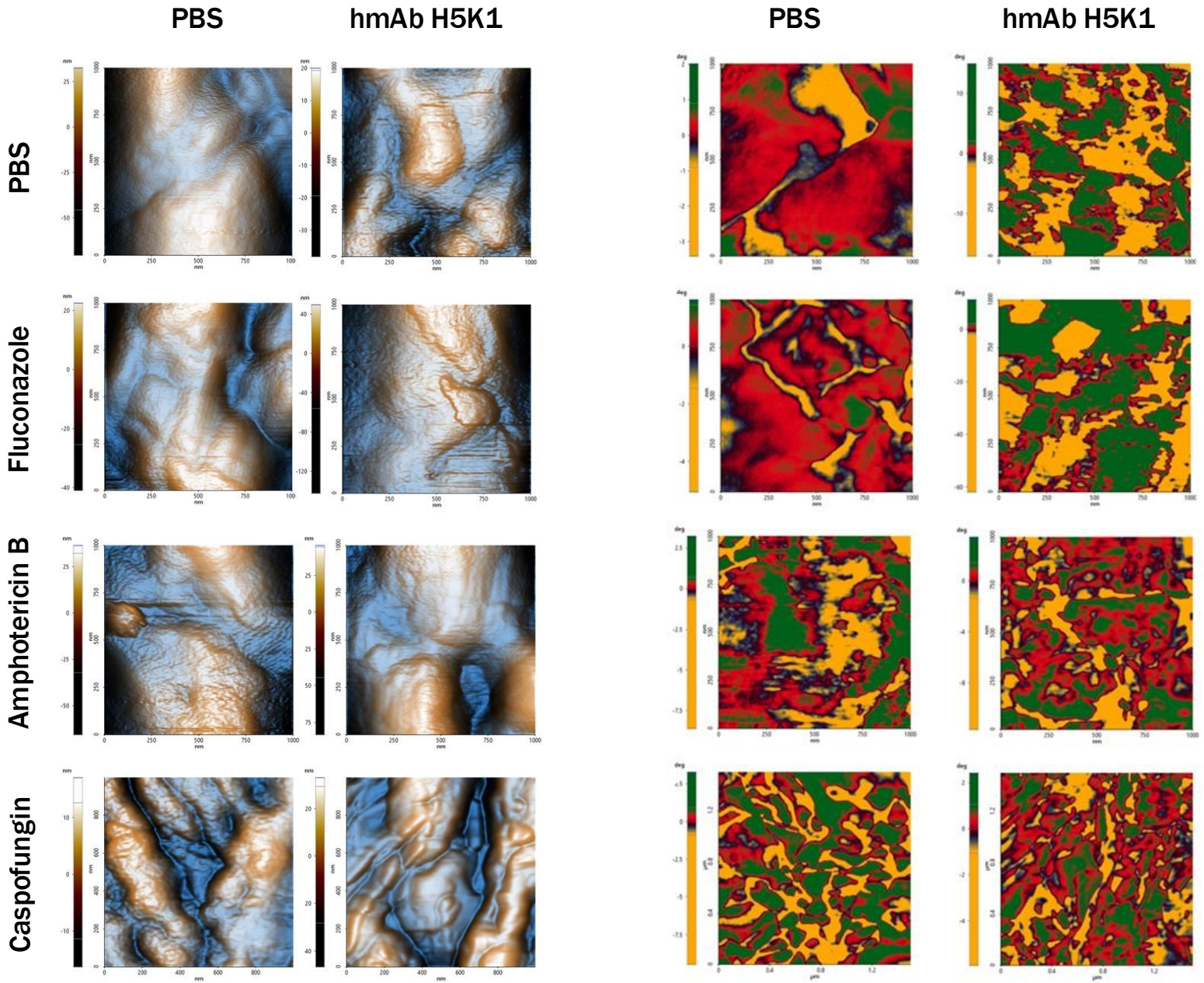
Red population: not-engulfing macrophages
Blue population: engulfing macrophages
Green population: residual *C. auris*











Atomic force microscopy

- Perturbation in the topographic and sub-topographic domains
- Higher frequency of vertical alterations
- Substantial difference in the chemical distribution
- Softening and weakening of the whole cell

Dia-T51 - effect in combination - *C. auris*

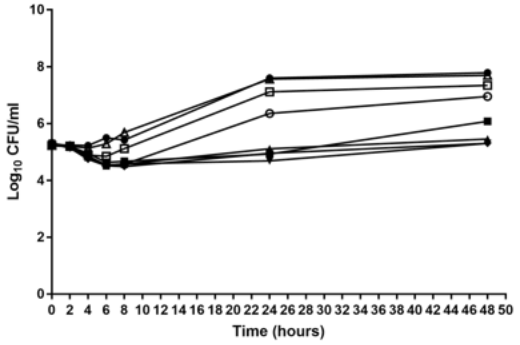
Dia-T51 + Fluconazole

Dia-T51 µg/ml	0	0.25	2.5	25	250
24h MIC FLU µg/ml	2	2	2	2	2
48h MIC FLU µg/ml	4	4	4	4	2

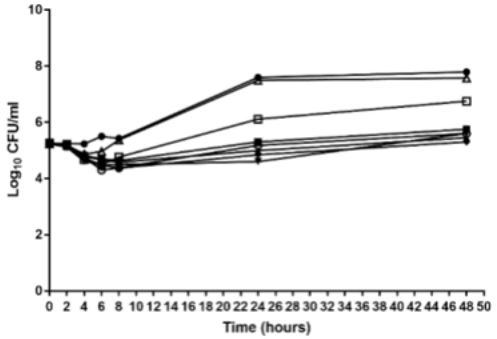
Dia-T51 + Caspofungin

Dia-T51 µg/ml	0	0.25	2.5	25	250
24h MIC CAS µg/ml	0.0625	0.0625	0.0625	0.0625	0.0625
48h MIC CAS µg/ml	0.25	0.125	0.125	0.125	0.125

Caspofungin



Dia-T51 + Caspofungin

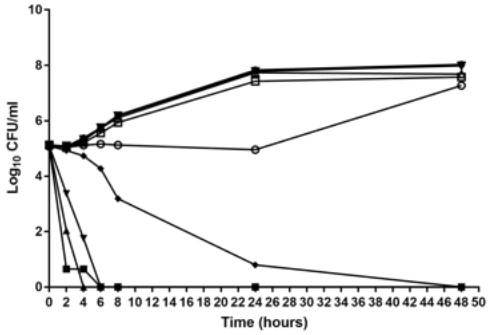


Synergic per definition

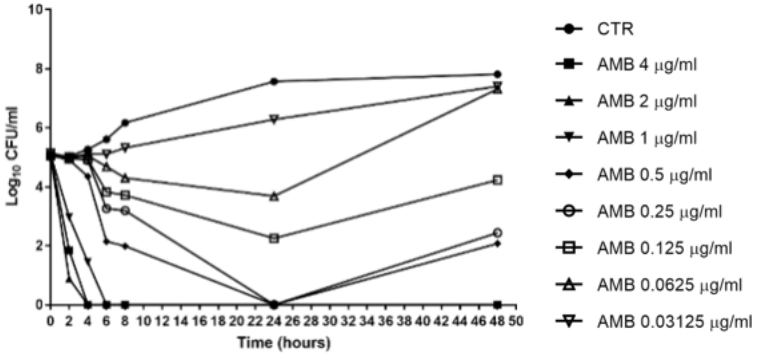
Dia-T51 + Amphotericin B

Dia-T51 µg/ml	0	0.25	2.5	25	250
24h MIC AMB µg/ml	0.5	0.5	0.25	0.125	0.125
48h MIC AMB µg/ml	1	1	1	0.25	0.25

Amphotericin B



Dia-T51 + Amphotericin B

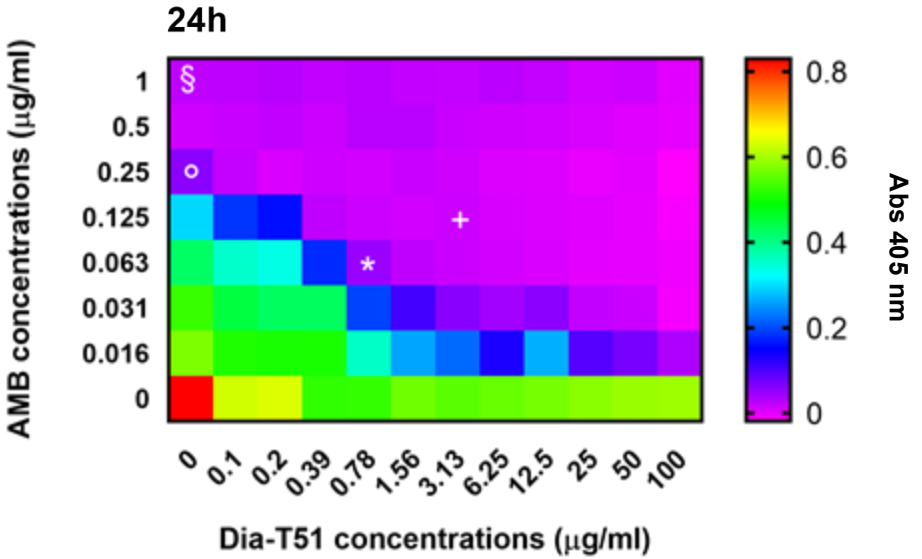


Synergic

- Is it synergic at lower concentrations?
- Is it effective with other *Candida* spp.?
- Is it efficient with resistant strains?

4 clinical isolates of *C. glabrata*

Dia-T51 effect in combination - *C. auris*

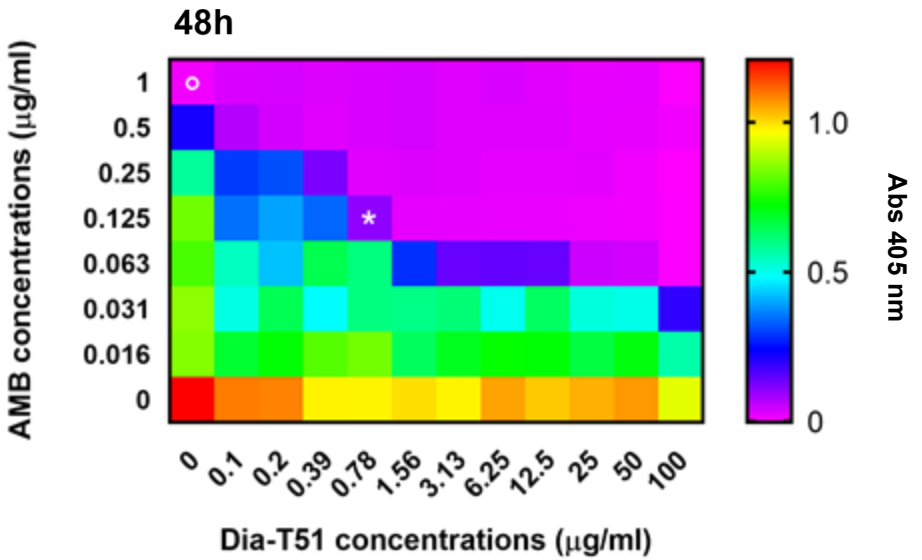


○ MIC AMB

* Synergic MIC Dia-T51+AMB (FICI: 0.256)

§ MFC AMB

\+ MFC Dia-T51+AMB

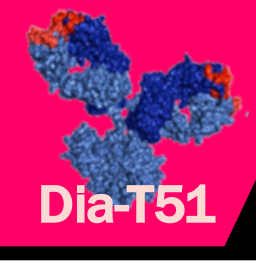


○ MIC AMB

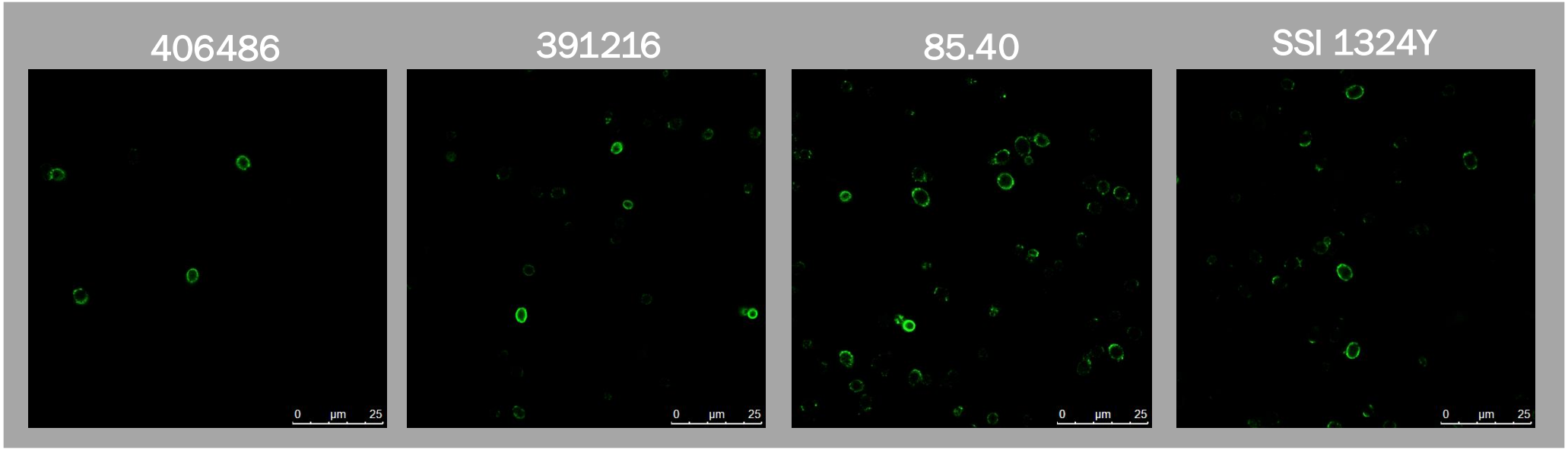
* Synergic MIC Dia-T51+AMB (FICI: 0.129)

Minimum Inhibitory Concentration (inhibition of fungal growth ≥90%)	**24h**	**48h**
AMB	0.25 µg/ml	1 µg/ml
Dia-T51+AMB	0.78+0.063 µg/ml	0.78+0.125 µg/ml

Minimum Fungicidal Concentration (reduction of initial inoculum viability ≥99.9%)	**AMB**	**Dia-T51+AMB**
	1 µg/ml	3.13+0.125 µg/ml

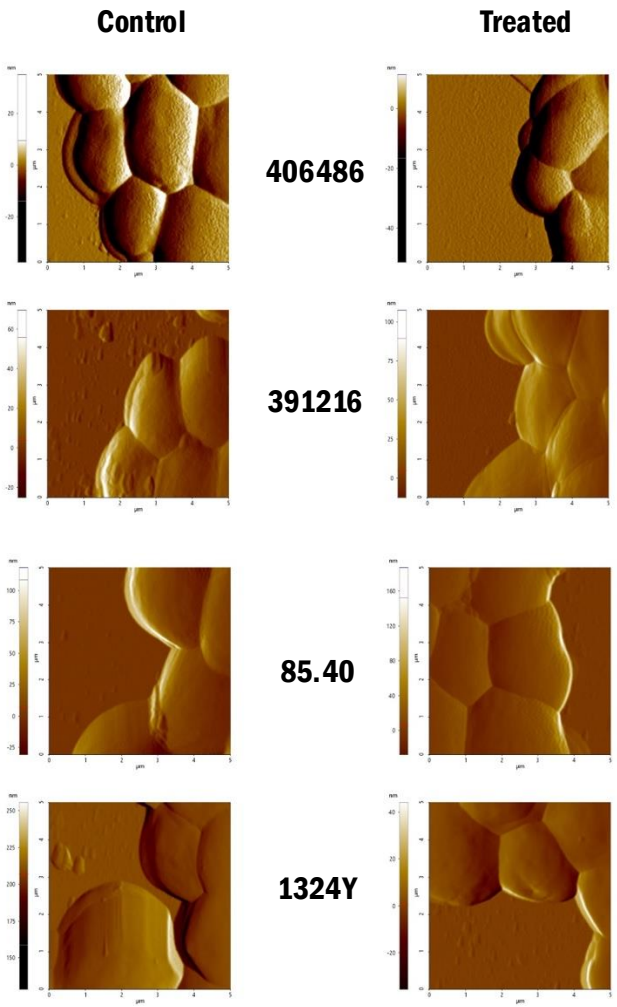


Dia-T51 effect in combination - *C. glabrata*

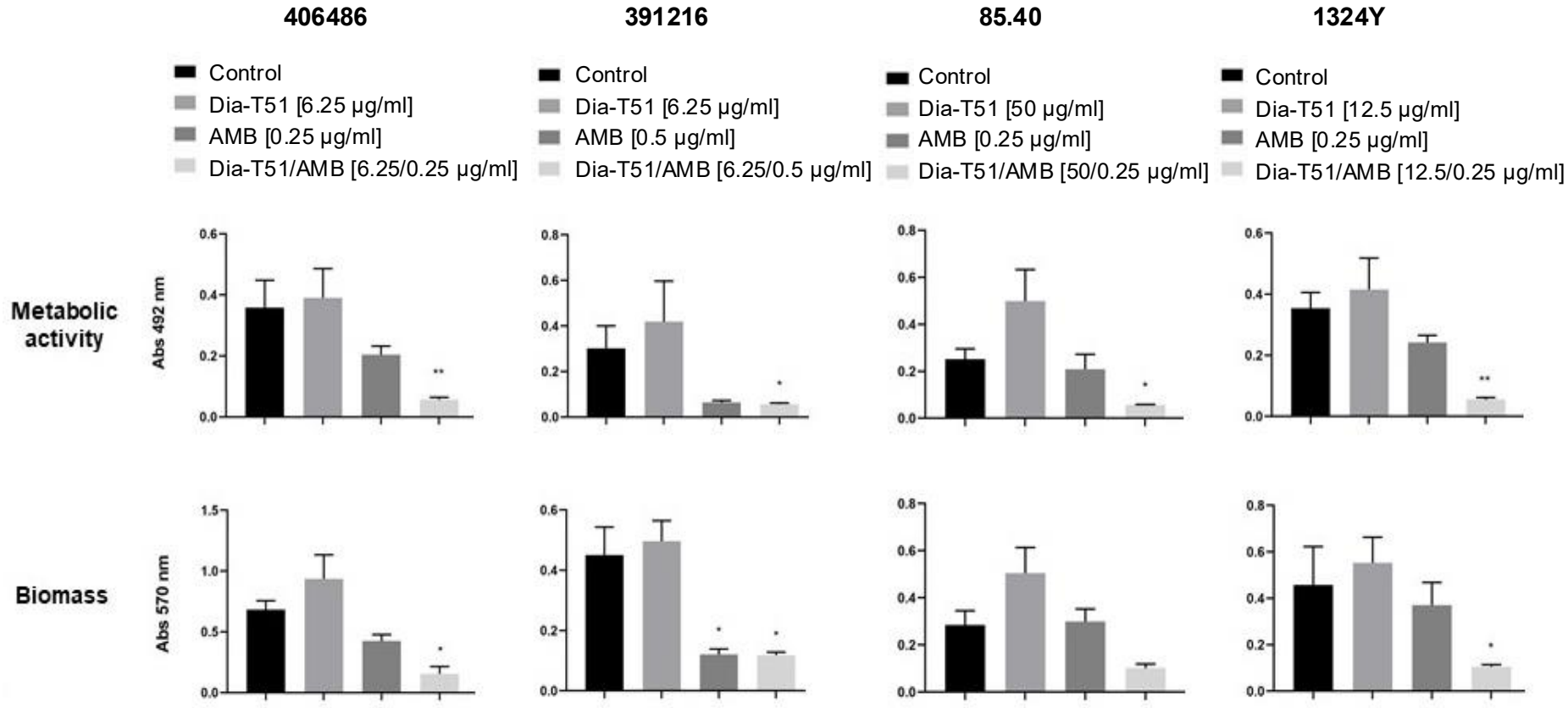


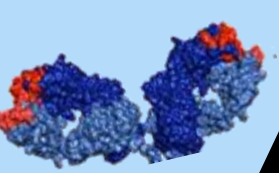
		Synergy in Checkerboard		Synergy in time-kill curves
***C. glabrata* strains**	**Characteristics**	**Dia-T51 / AMB (µg/ml)**	**FICI**	**Δlog most active compound vs combination ≥ 2 Dia-T51 / AMB (µg/ml)**
406486	Biofilm hyperproducer	0.78 / 0.125	0.252	6.25/0.25
391216	Common clinical isolate	1.56 / 0.25	0.254	6.25/0.5
85.40	Mutation in FKS gene	12.5 / 0.125	0.281	50/0.25
1324Y	Mutation in FKS gene	3.13 / 0.125	0.258	12.5/0.25

Dia-T51 effect in combination - *C. glabrata*

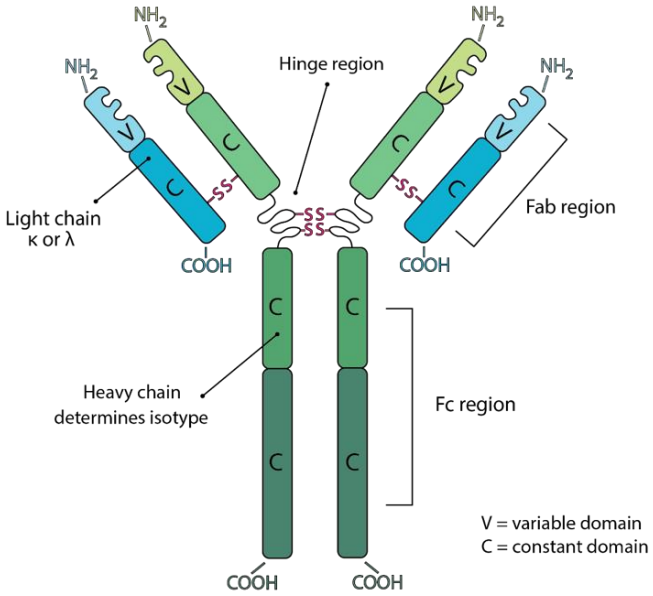


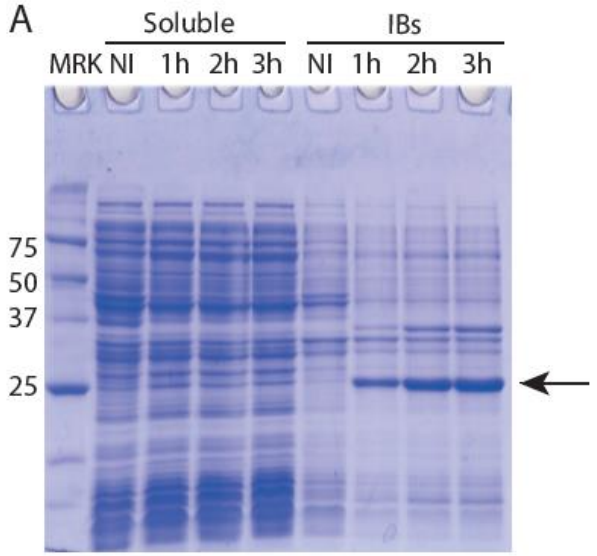
Anti-biofilm activity

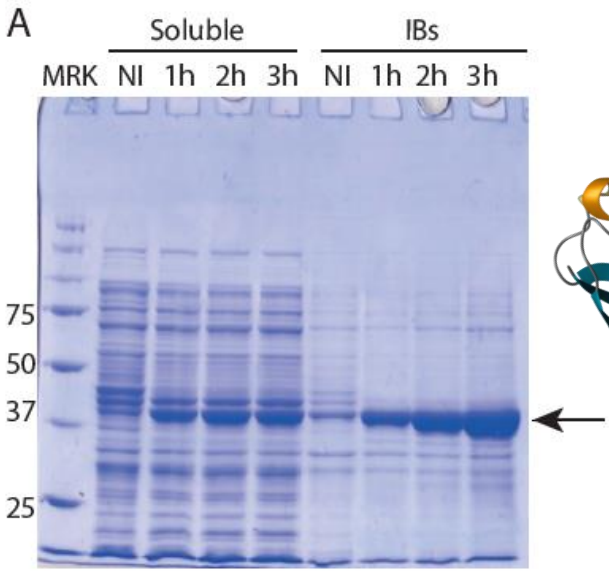


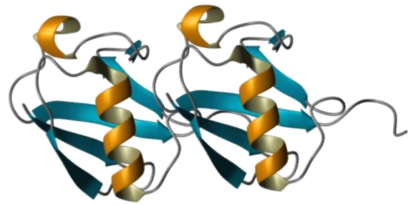


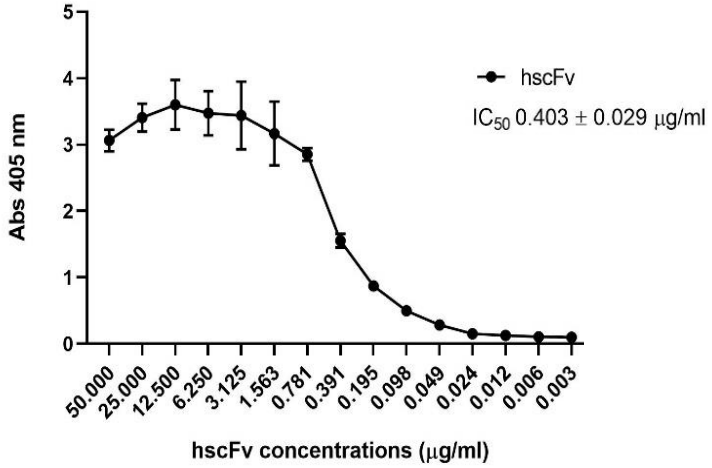
ScFv-3T





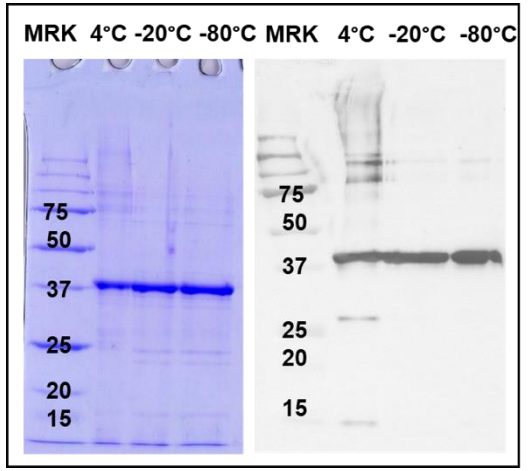


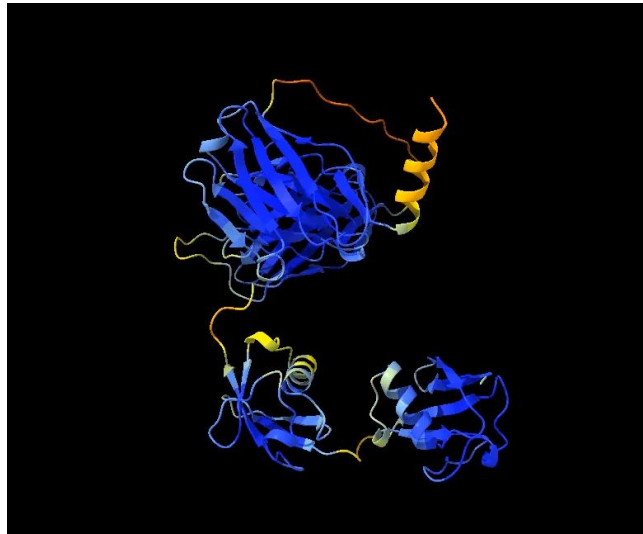


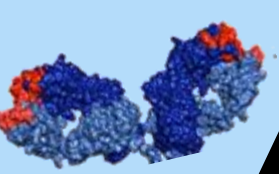


Time: 3 months

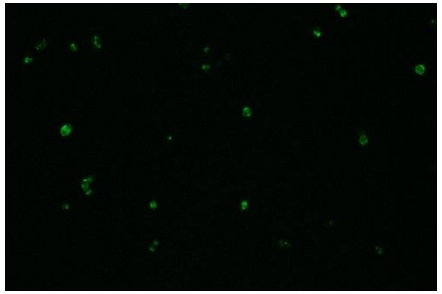
Temperatures	IC_{50} µg/ml
4°C	0.74
-20°C	0.44
-80°C	0.33



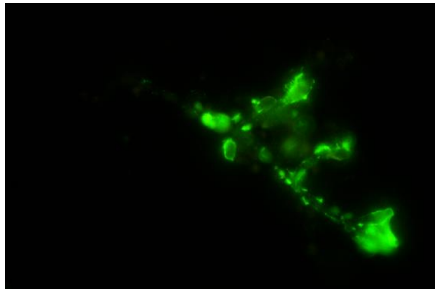




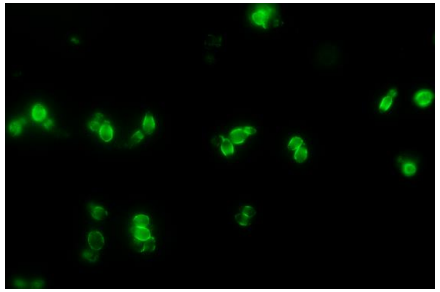
ScFv-3T alone and in combination



C. auris

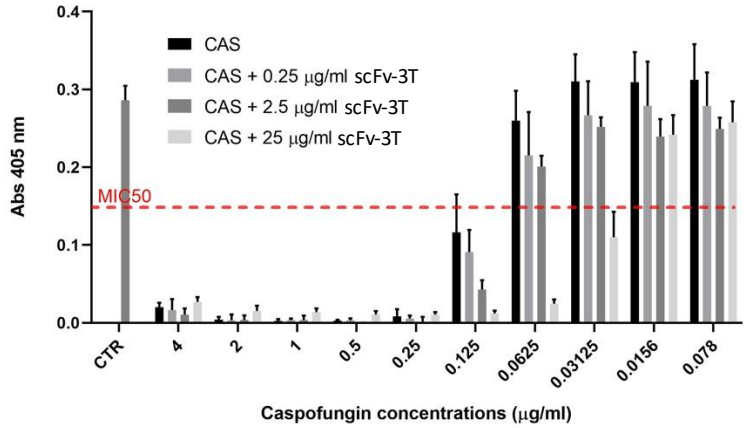


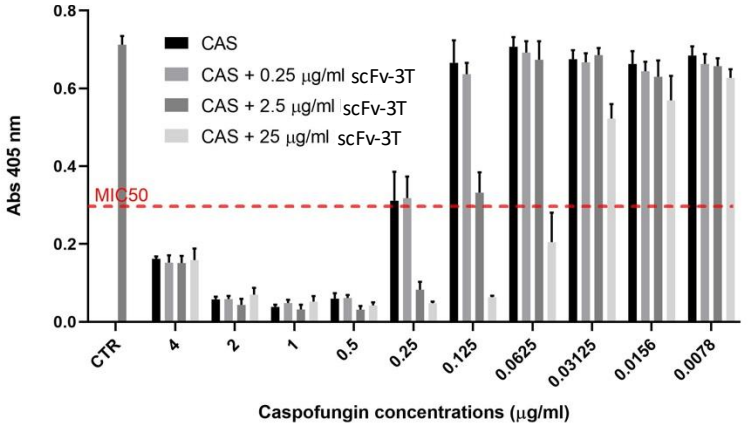
C. albicans hyphal form



C. albicans yeast form

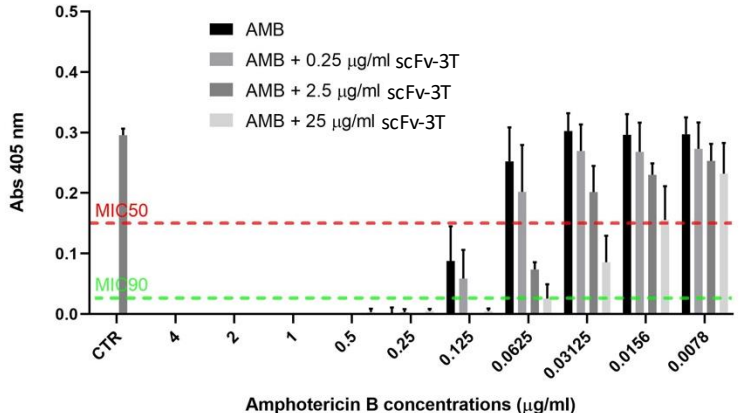
scFv-3T + Caspofungin

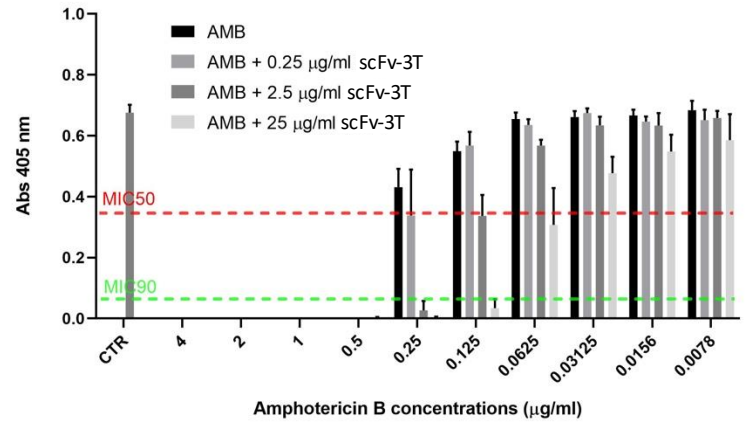




scFv-3T µg/ml	0	0.25	2.5	25
24h MIC CAS µg/ml	0.125	0.125	0.125	0.03125
48h MIC CAS µg/ml	0.25	0.25	0.125	0.0625

scFv-3T + Amphotericin B





scFv-3T µg/ml	0	0.25	2.5	25
24h MIC AMB µg/ml	0.25	0.25	0.125	0.0625
48h MIC AMB µg/ml	0.5	0.5	0.25	0.125

1506 UNIVERSITÀ DEGLI STUDI DI URBINO CARLO BO | DISB DIPARTIMENTO DI SCIENZE BIOMOLECOLARI

DIATHEVA

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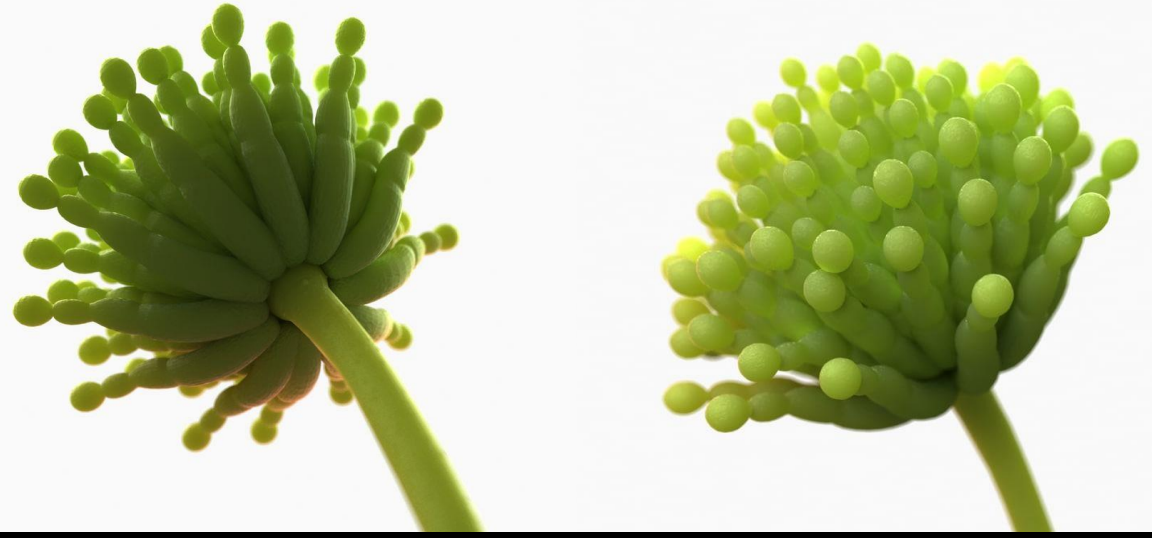
(grant ECS00000041 - VITALITY - CUP H33C22000430006)



Thank you!

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CONCLUSIONS & FUTURE PERSPECTIVES

Deepen some aspects

- Dia-T51 and scFv-3T are the humanized monoclonal antibodies based on the murine mAb 2G8.
- Both antibodies have been widely characterized *in vitro*.

Explore the intracellular pathways

- They bind efficently β-1,3-glucans on different fungal species.

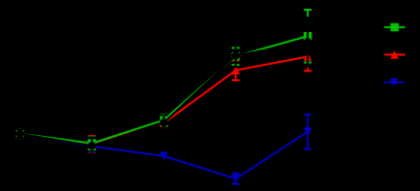
Investigate the relationship with the host immune system

Focus on *in vivo* studies

Create targeted delivery systems

Dia-T51 could be a new leading biological drug against pathogenic fungi. We are optimistic in its advancement to clinical phases.

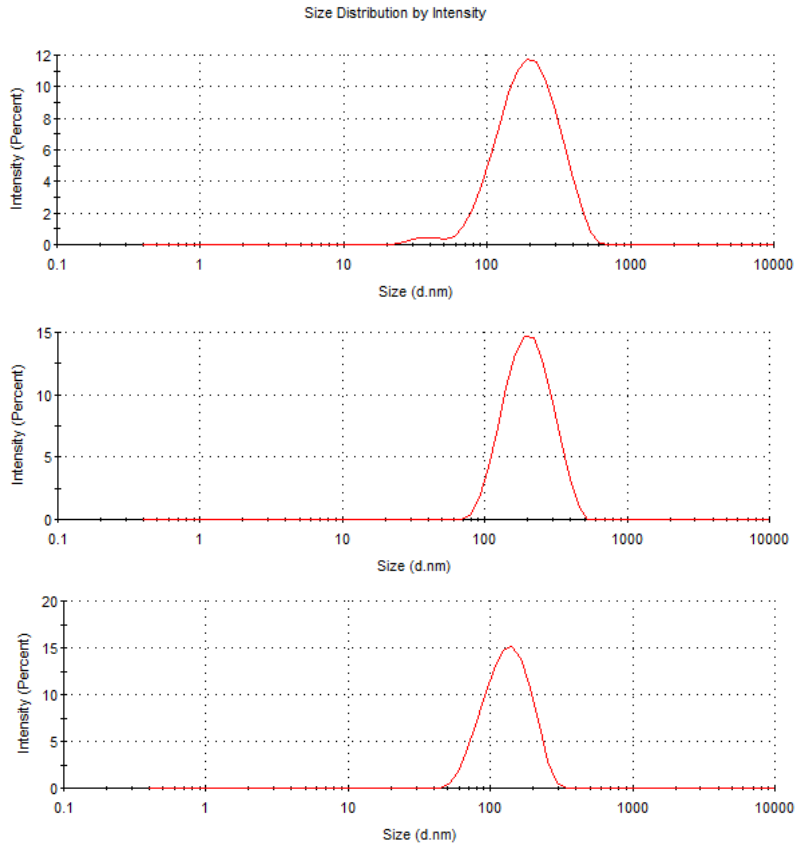
scFv-3T must be further explored but has already demonstrated promising results.



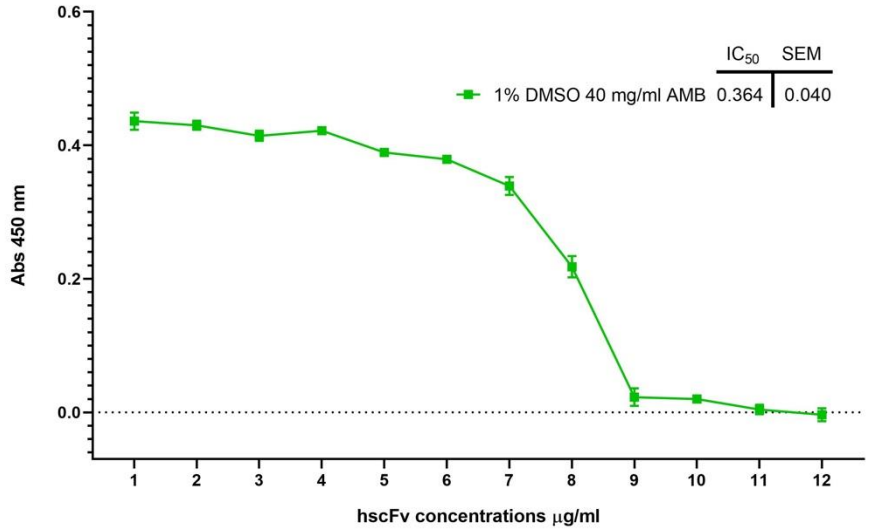
Targeted delivery systems: HOW?

Sample	Z-Average (nm)	PdI
Ub Monomer	1233.8	0.79
Ub Dimer	93.1	0.19
Ub Trimer	231.2	0.36
hscFv-3T **(Ub Dimer + humanized scFv)**	179.4	0.22

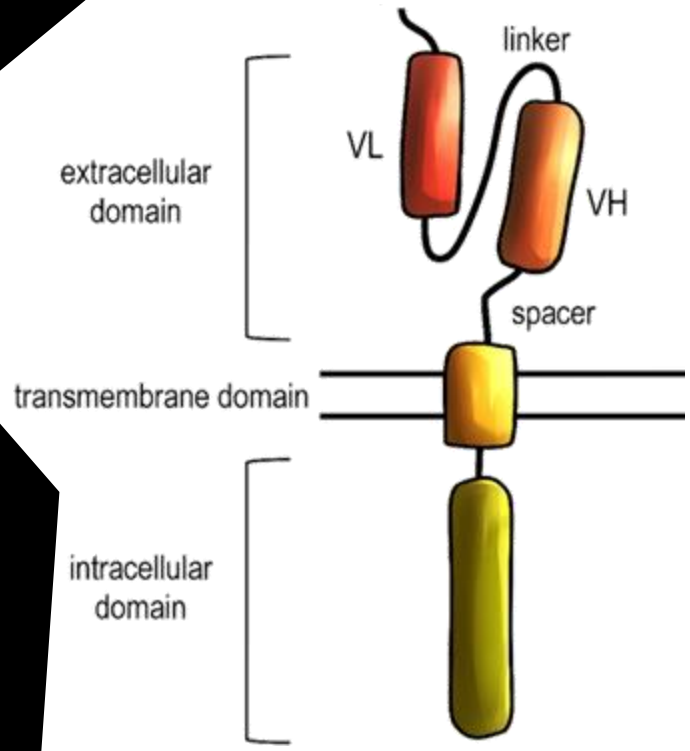
Z-Average: accepted diameter < 200 nm
PdI: accepted value < 0.25



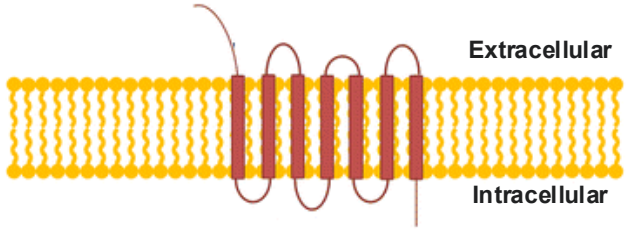
Loading of AMB in scFv-3T nanoparticles

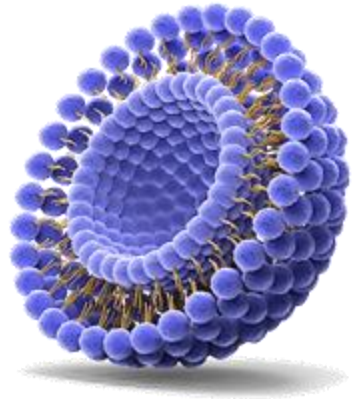


The DARC side of the MOON

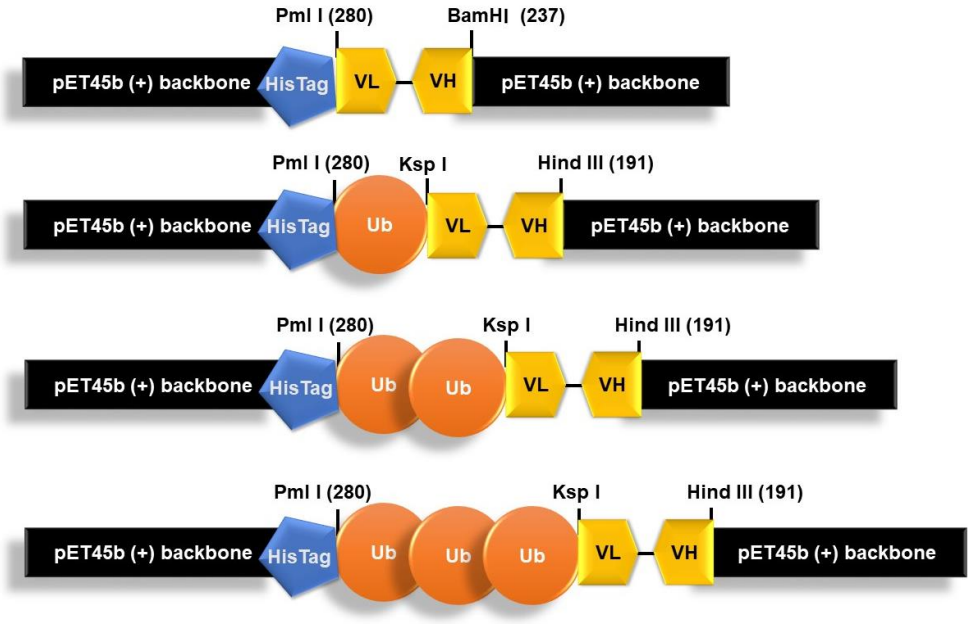


DARC protein
(Duffy antigen/chemokine receptor)



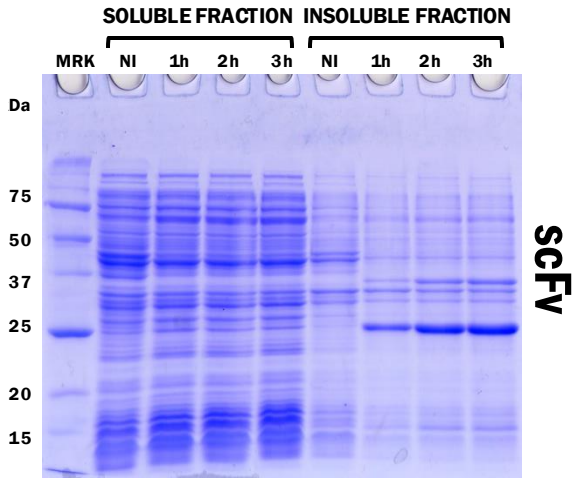


TAT penetrating peptide

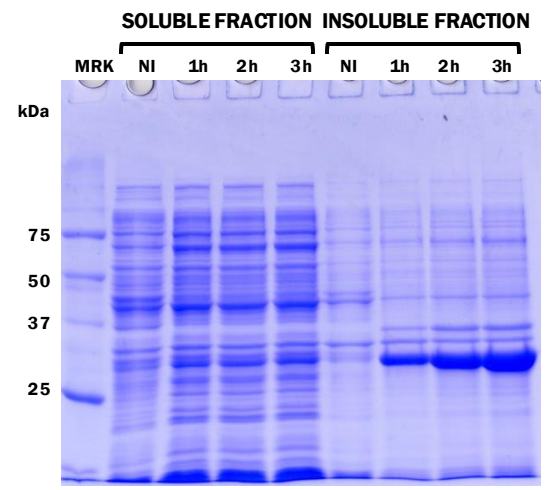


Why ubiquitin?

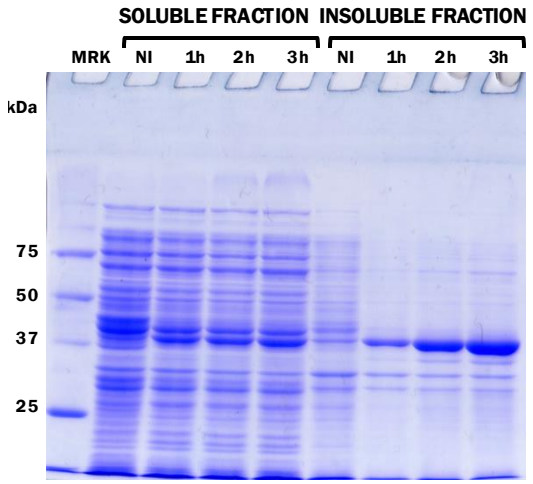
- Improves protein expression
- Helps the correct folding
- Increases solubility and stability
- Protects from proteolytic degradation
- Not immunogenic
- Polymerizes



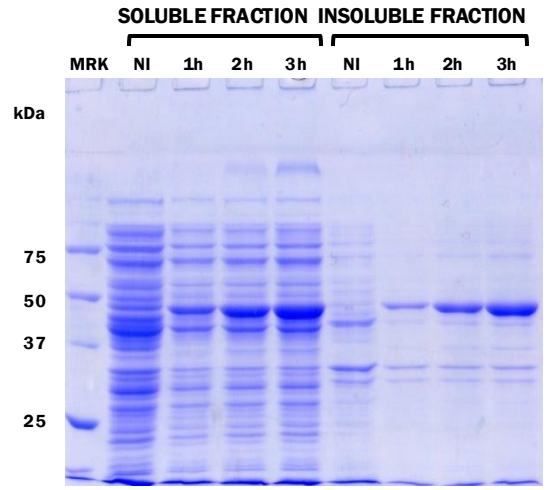
scFv



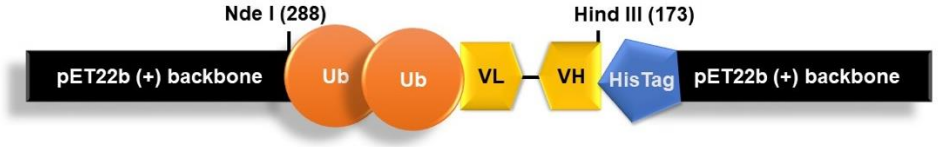
Ub monomer - scFv

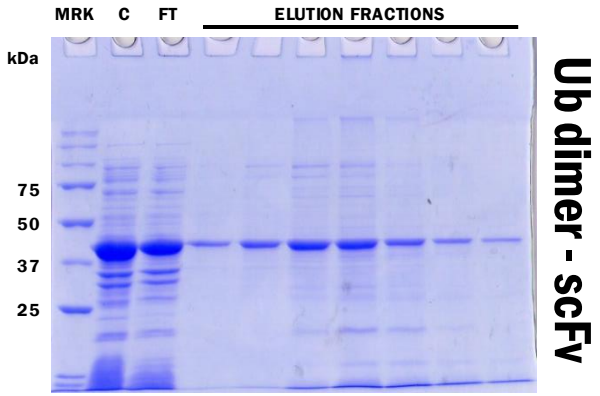


Ub dimer - scFv

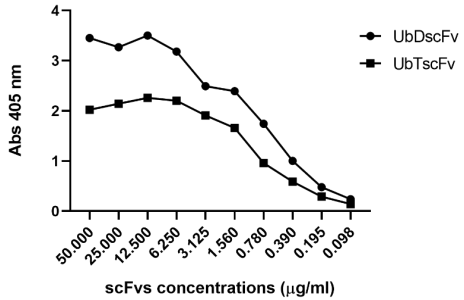


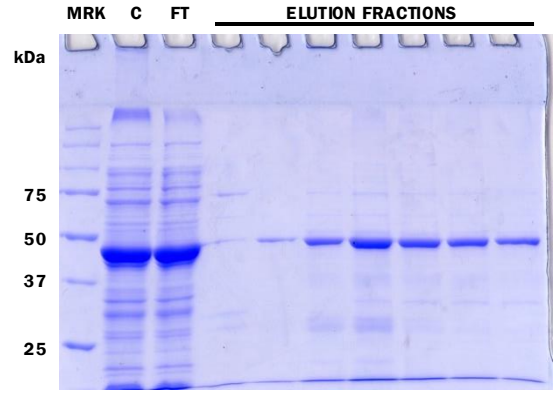
Ub trimer - scFv



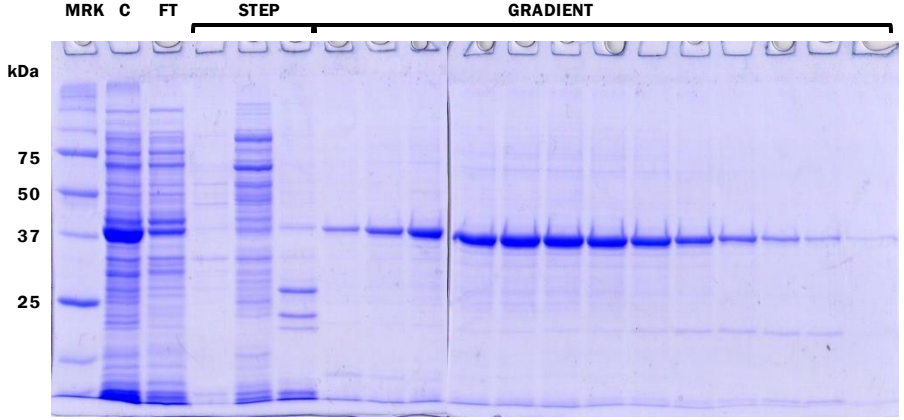


Ub dimer - scFv

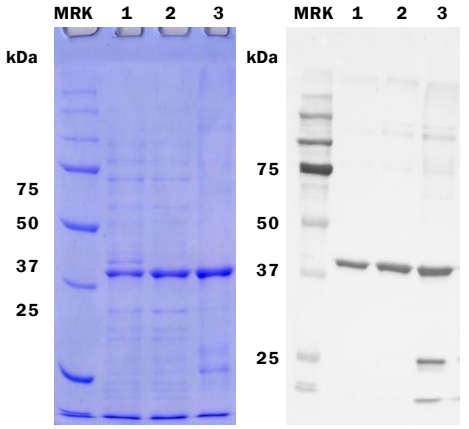




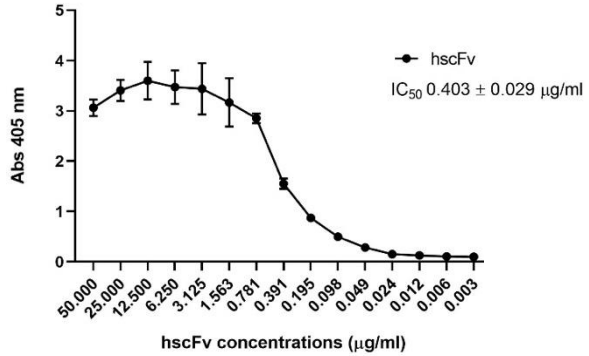
Ub trimer - scFv

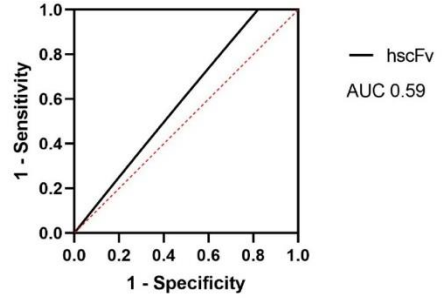


~40 mg from 1L

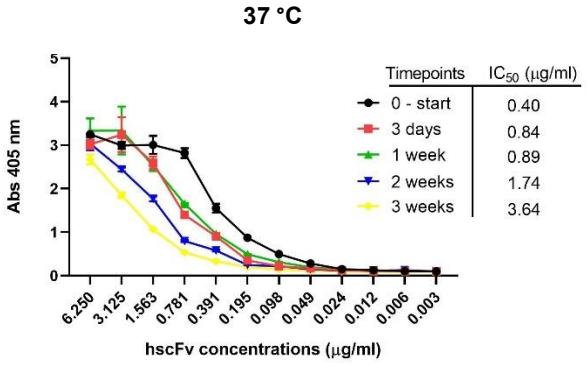


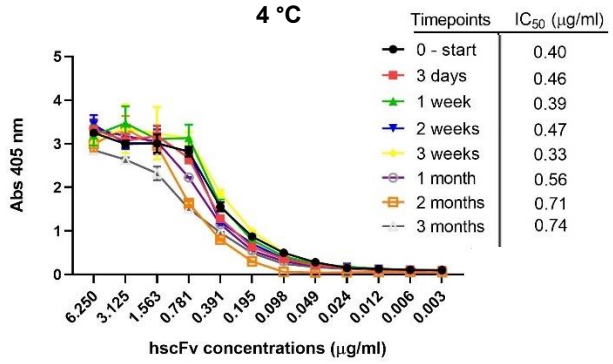
1 - Soluble fraction charged in Q Sepharose FF
2 - Negative passage in Q Sepharose
3 - Positive passage in Ni^{+2} Sepharose HP

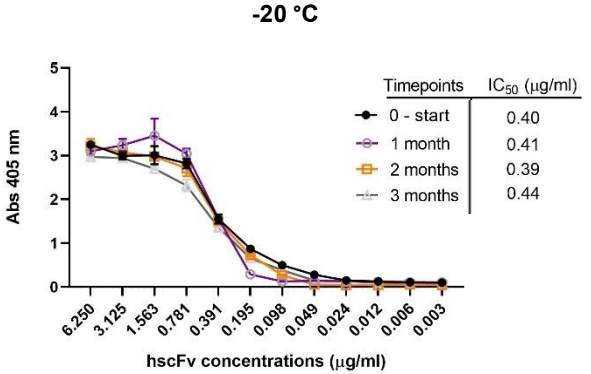


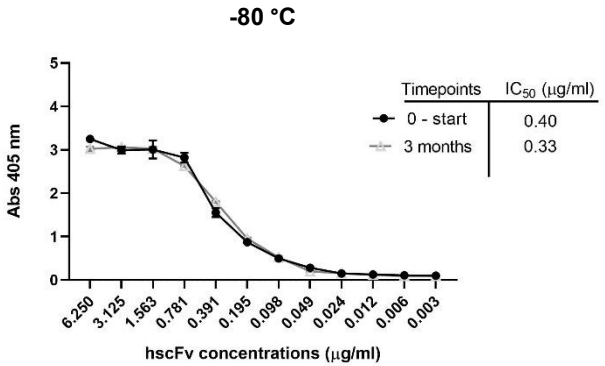


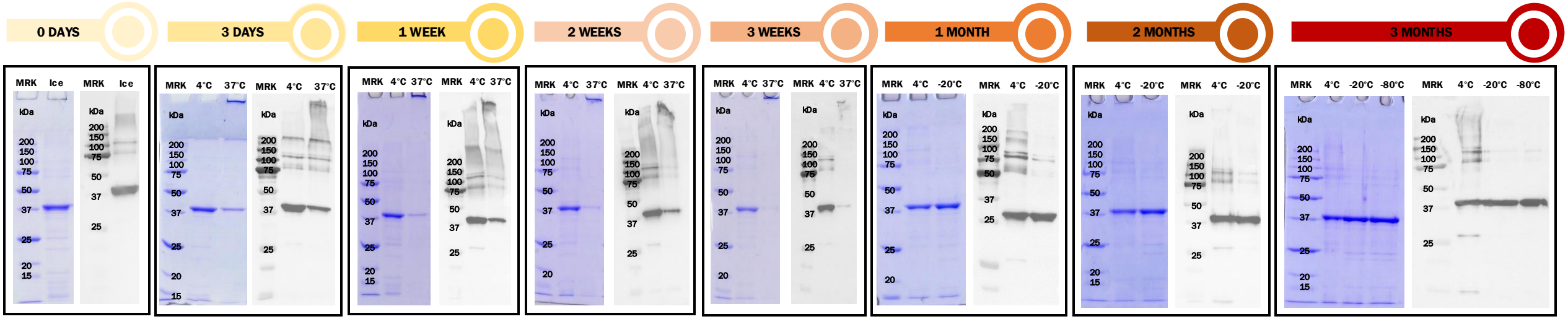
Stability test



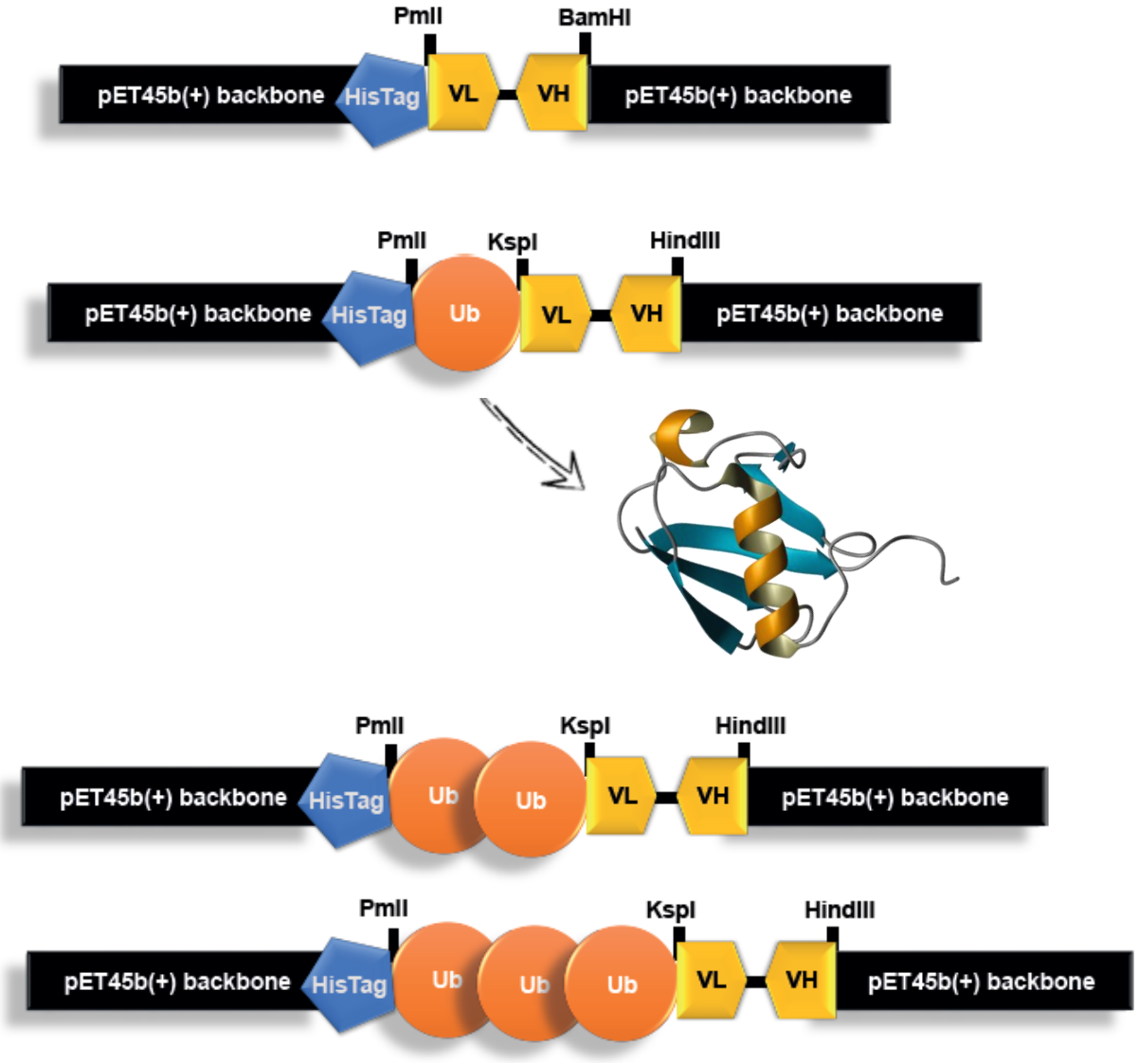








scFv-3T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BamHI
pET45b(+) backbone
HisTag
VL
VH
pET45b(+) backbone
PmlI
KspI
HindIII
pET45b(+) backbone
HisTag
Ub
VL
VH
pET45b(+) backbone
PmlI
KspI
HindIII
pET45b(+) backbone
HisTag
Ub
Ub
VL
VH
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HisTag
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Ub
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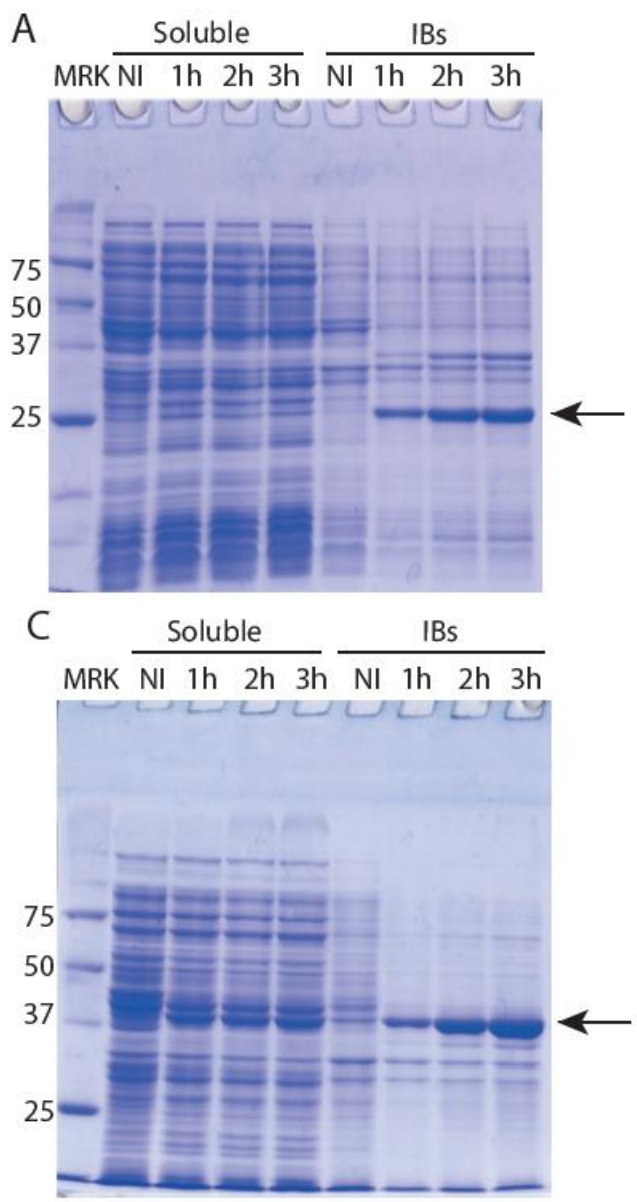
VH
pET45b(+) backbone


A
Soluble
IBs
MRK NI 1h 2h 3h NI 1h 2h 3h
75
50
37
25
B
Soluble
IBs
MRK NI 1h 2h 3h NI 1h 2h 3h
75
50
37
25
C
Soluble
IBs
MRK NI 1h 2h 3h NI 1h 2h 3h
75
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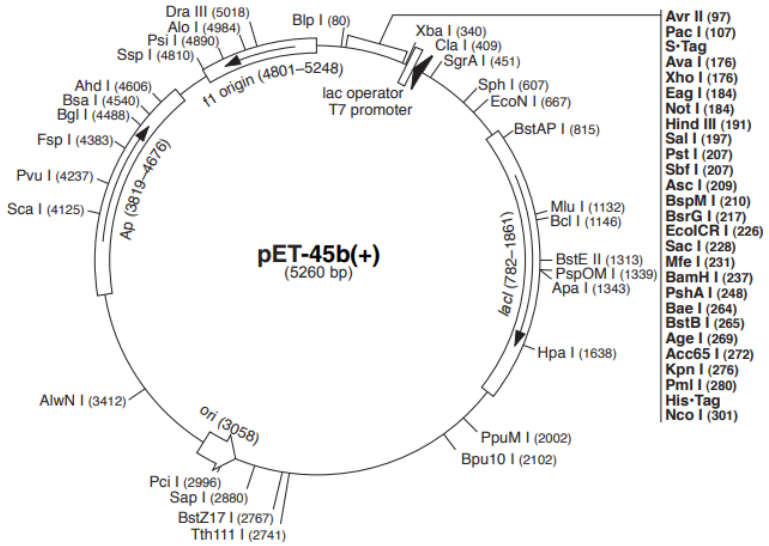
37
25
D
Soluble
IBs
MRK NI 1h 2h 3h NI 1h 2h 3h
75
50
37
25

scFv-3T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(5260 bp)
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lac operator
T7 promoter
lacI (782–1861)
Ap (3819–4676)
ori (3058)
Dra III (5018)
Alo I (4984)
Psi I (4890)
Ssp I (4810)
Blp I (80)
Xba I (340)
Cla I (409)
SgrA I (451)
Sph I (607)
EcoN I (667)
BstAP I (815)
Mlu I (1132)
Bcl I (1146)
BstE II (1313)
PspOM I (1339)
Apa I (1343)
Hpa I (1638)
PpuM I (2002)
Bpu10 I (2102)
Pci I (2996)
Sap I (2880)
BstZ17 I (2767)
Tth111 I (2741)
AlwN I (3412)
Ahd I (4606)
Bsa I (4540)
Bgl I (4488)
Fsp I (4383)
Pvu I (4237)
Sca I (4125)
Avr II (97)
Pac I (107)
S·Tag
Ava I (176)
Xho I (176)
Eag I (184)
Not I (184)
Hind III (191)
Sal I (197)
Pst I (207)
Sbf I (207)
Asc I (209)
BspM I (210)
BsrG I (217)
EcoICR I (226)
Sac I (228)
Mfe I (231)
BamH I (237)
PshA I (248)
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Age I (269)
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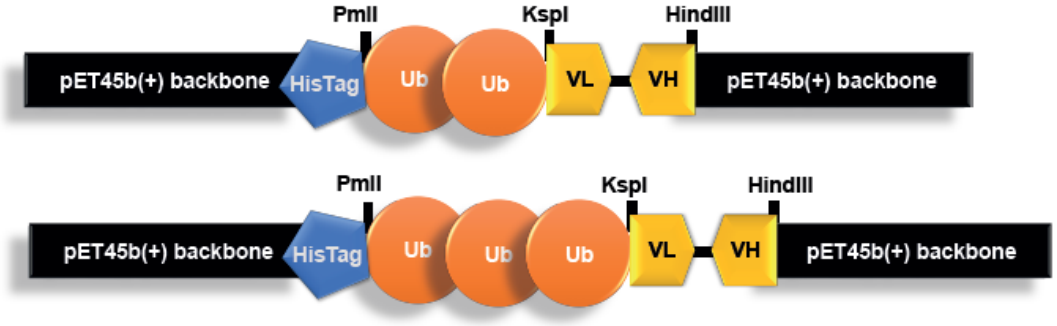
Kpn I (276)
Pml I (280)
His·Tag
Nco I (301)


PmlI
KspI
HindIII
pET45b(+) backbone
HisTag
Ub
Ub
VL
VH
pET45b(+) backbone
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KspI
HindIII
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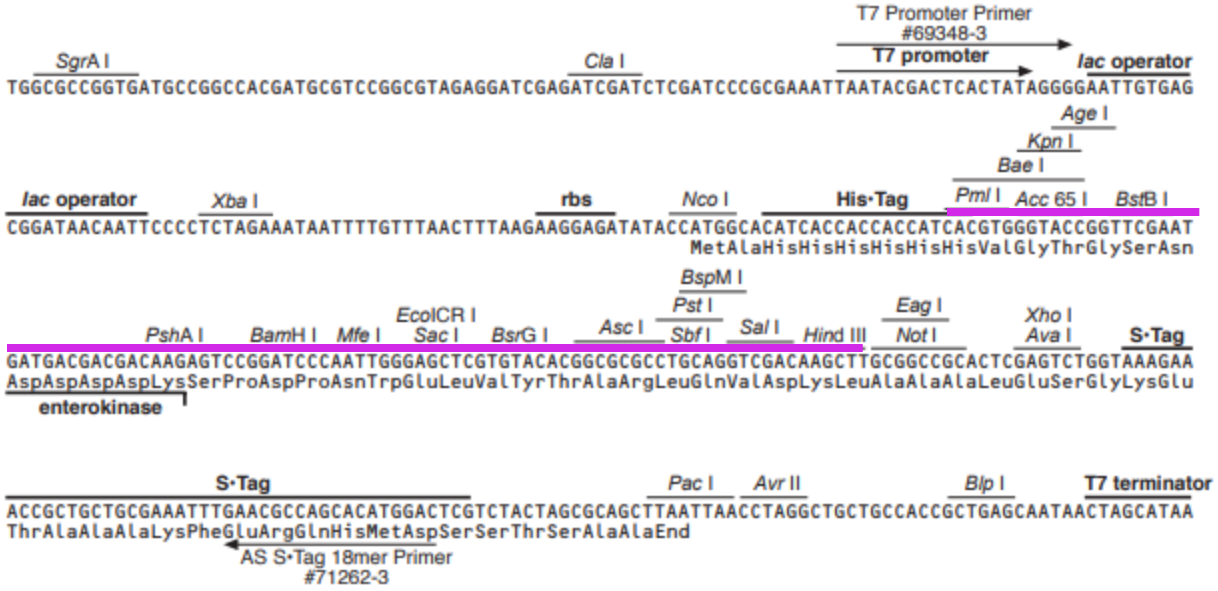
Ub
Ub
Ub
VL
VH
pET45b(+) backbone


T7 Promoter Primer
#69348-3
T7 promoter
SgrA I
Cla I
lac operator
TGGCGCCGGTGATGCCGGCCACGATGCGTCCGGCGTAGAGGATCGAGATCGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAG
Age I
Kpn I
Bae I
lac operator
Xba I
rbs
Nco I
His·Tag
Pml I
Acc 65 I
BstB I
CGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACCATGGCACATCACCACCACCATCACGTGGGTACCGGTTCGAAT
MetAlaHisHisHisHisHisHisValGlyThrGlySerAsn
BspM I
Pst I
EcoICR I
Eag I
Xho I
PshA I
BamH I
Mfe I
Sac I
BsrG I
Asc I
Sbf I
Sal I
Hind III
Not I
Ava I
S·Tag
GATGACGACGACAAGAGTCCGGATCCCAATTGGGAGCTCGTGTACACGGCGCGCCTGCAGGTCGACAAGCTTGCGGCCGCACTCGAGTCTGGTAAAGAA
AspAspAspAspLysSerProAspProAsnTrpGluLeuValTyrThrAlaArgLeuGlnValAspLysLeuAlaAlaAlaLeuGluSerGlyLysGlu
enterokinase
S·Tag
Pac I
Avr II
Blp I
T7 terminator
ACCGCTGCTGCGAAATTTGAACGCCAGCACATGGACTCGTCTACTAGCGCAGCTTAATTAACCTAGGCTGCTGCCACCGCTGAGCAATAACTAGCATAA
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AS S·Tag 18mer Primer
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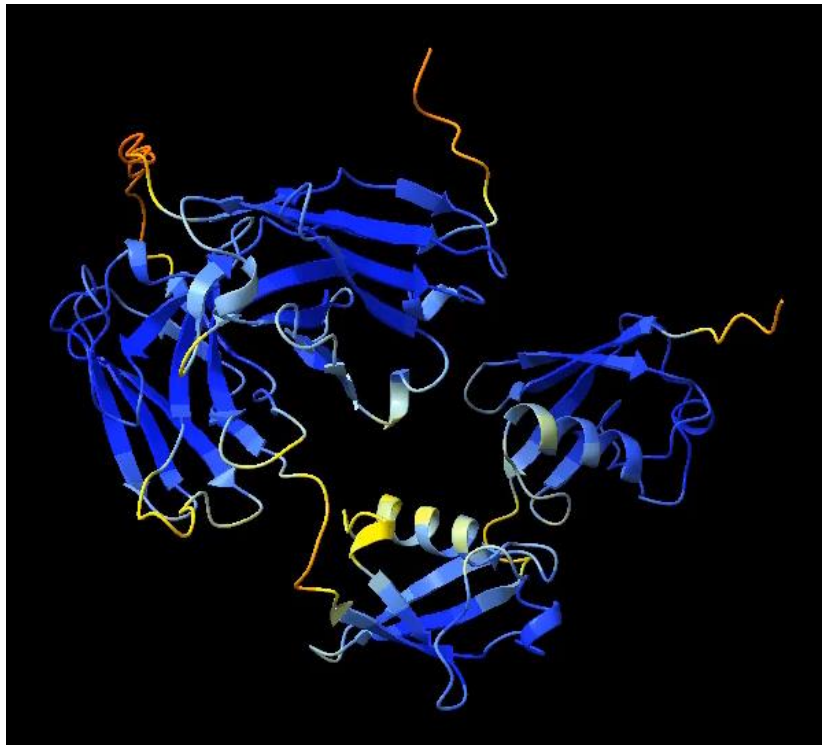
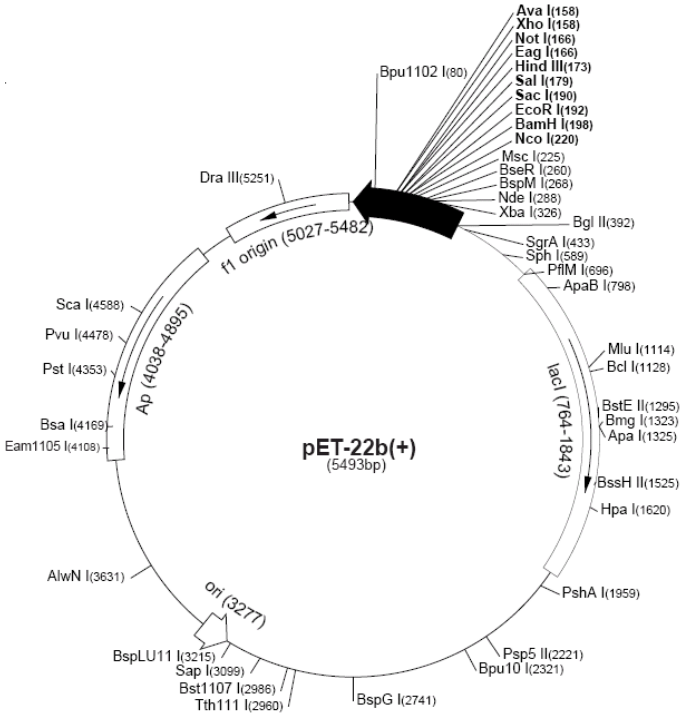


scFv-3T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Xho I(158)
Not I(166)
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Hind III(173)
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Sac I(190)
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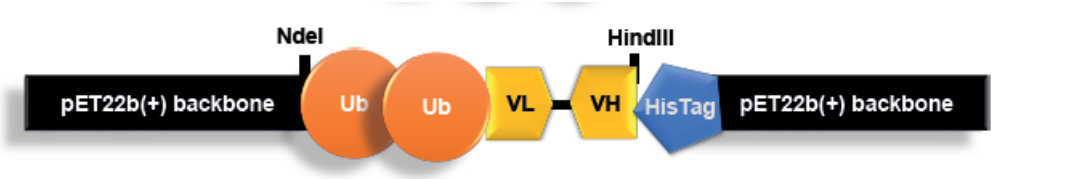
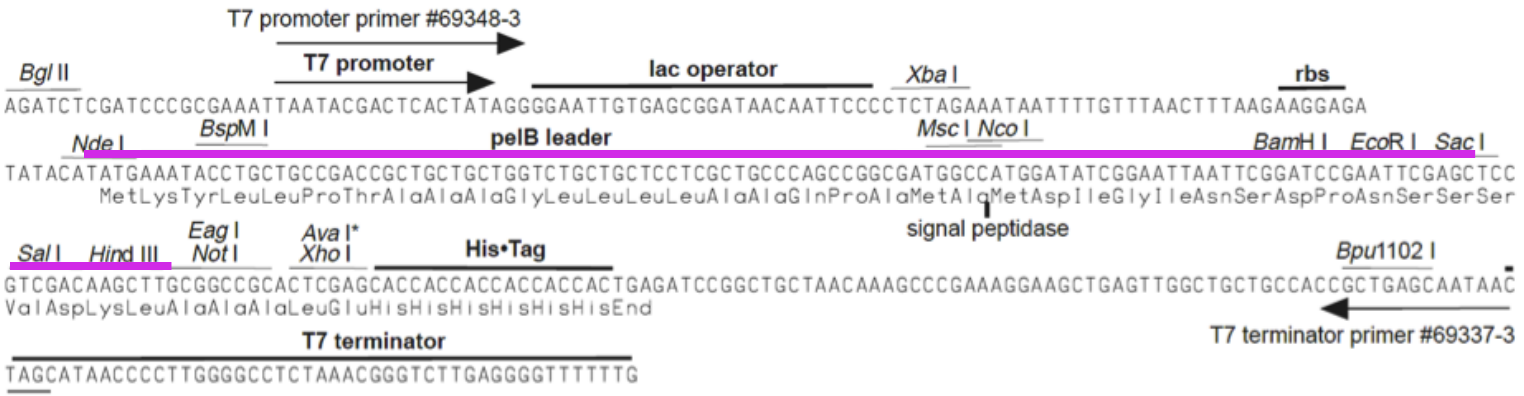
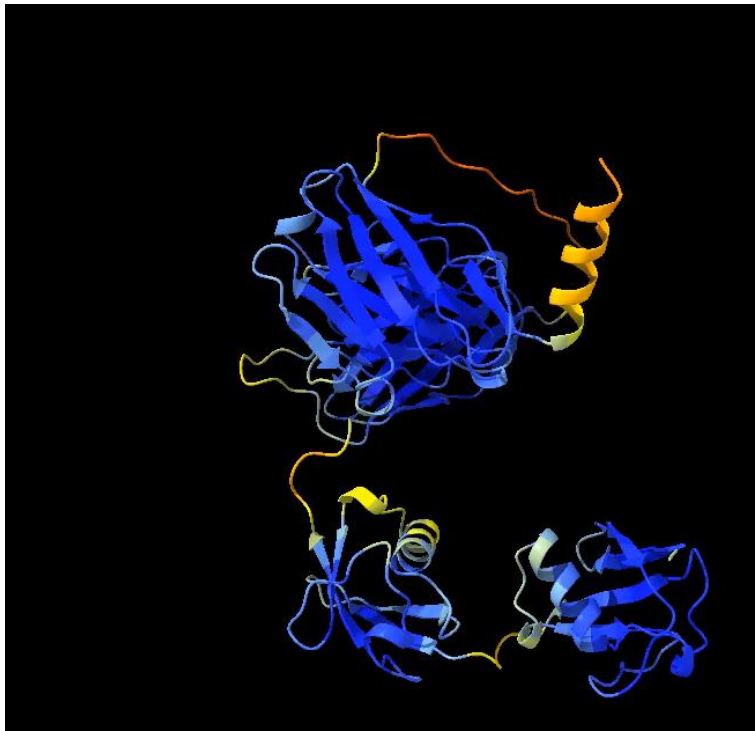
BamH I(198)
Nco I(220)
Msc I(225)
BseR I(260)
BspM I(268)
Nde I(288)
Xba I(326)
Bpu1102 I(80)
Bgl II(392)
SgrA I(433)
Sph I(589)
PflM I(696)
ApaB I(798)
Mlu I(1114)
Bcl I(1128)
BstE II(1295)
Bmg I(1323)
Apa I(1325)
BssH II(1525)
Hpa I(1620)
PshA I(1959)
Psp5 II(2221)
Bpu10 I(2321)
BspG I(2741)
Bst1107 I(2986)
Tth111 I(2960)
Sap I(3099)
BspLU11 I(3215)
AlwN I(3631)
Eam1105 I(4108)
Bsa I(4169)
Pst I(4353)
Pvu I(4478)
Sca I(4588)
Dra III(5251)
pET-22b(+)
(5493bp)
f1 origin (5027-5482)
Ap (4038-4895)
lacI (764-1843)
ori (3277)

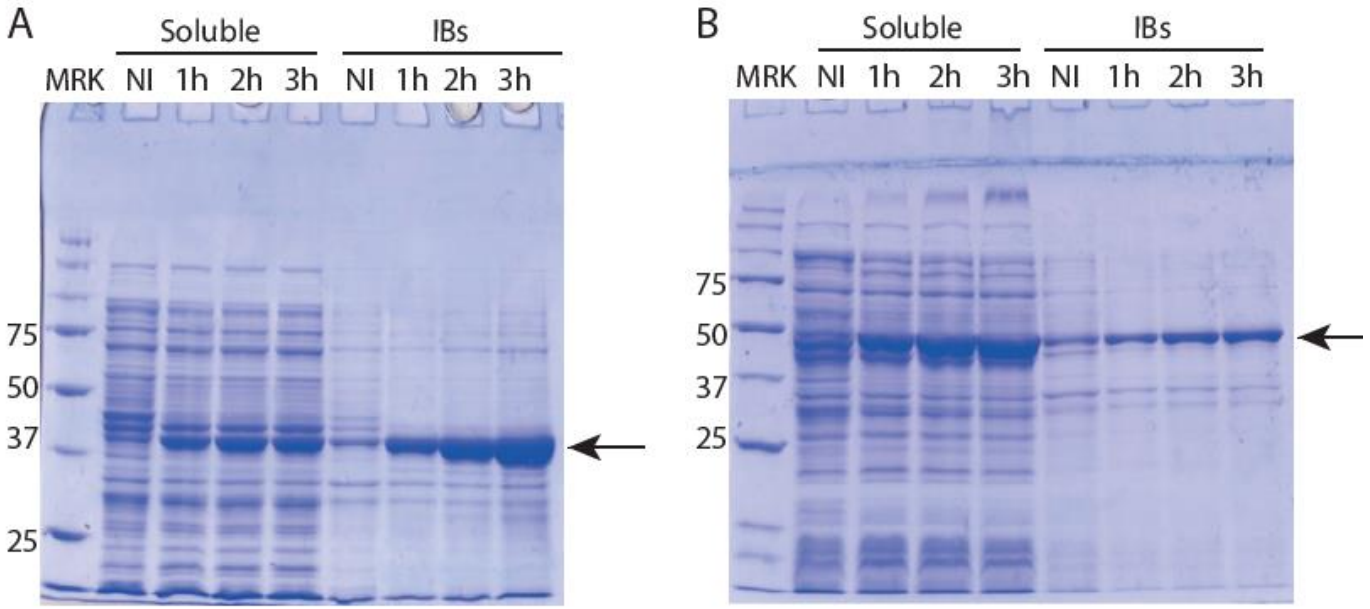

NdeI
HindIII
pET22b(+) backbone
Ub
Ub
VL
VH
HisTag
pET22b(+) backbone

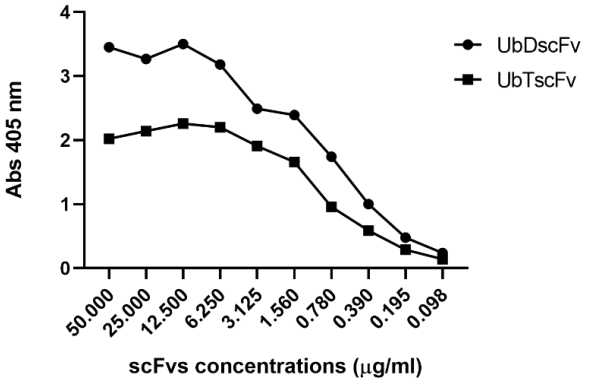

NdeI
HindIII
pET22b(+) backbone
Ub
Ub
Ub
VL
VH
HisTag
pET22b(+) backbone

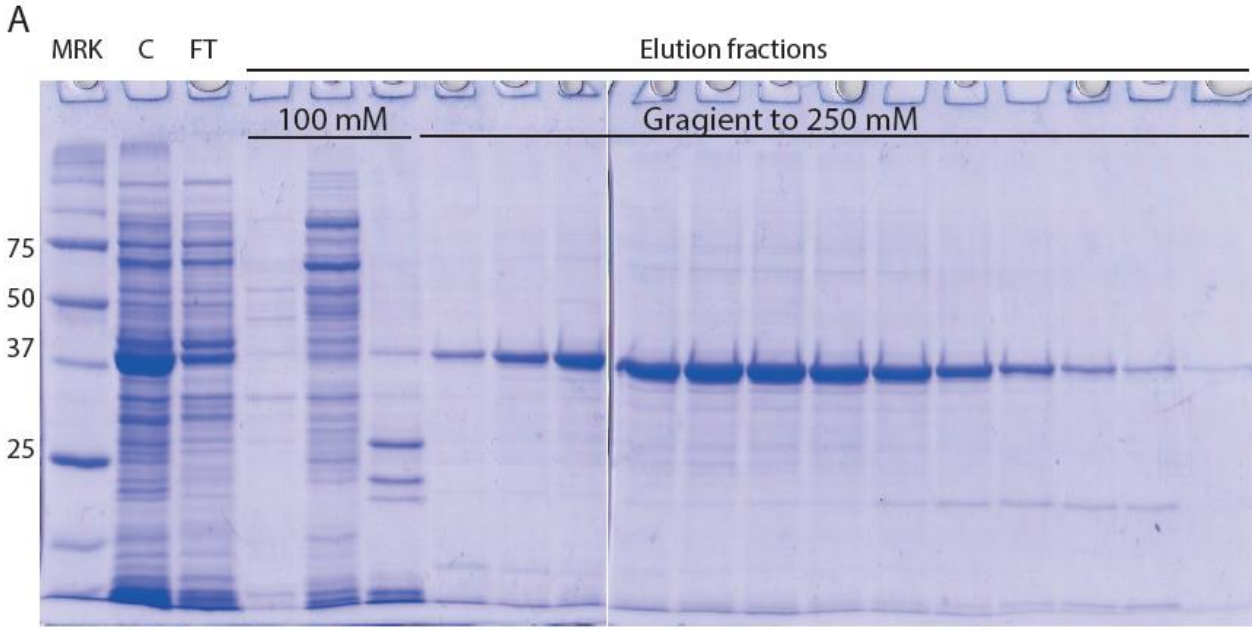

T7 promoter primer #69348-3
T7 promoter
lac operator
Bgl II
Xba I
rbs
AGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGA
Nde I
BspM I
pelB leader
Msc I Nco I
BamH I EcoR I Sac I
TATACATATGAAATACCTGCTGCCGACCGCTGCTGCTGGTCTGCTGCTCCTCGCTGCCCAGCCGGCGATGGCCATGGATATCGGAATTAATTCGGATCCGAATTCGAGCTCC
MetLysTyrLeuLeuProThrAlaAlaAlaGlyLeuLeuLeuAlaAlaGlnProAlaMetAlaMetAspIleGlyIleAsnSerAspProAsnSerSerSer
signal peptidase
Sal I
Hind III
Eag I
Not I
Ava I*
Xho I
His•Tag
Bpu1102 I
GTCGACAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCACTGAGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAAC
ValAspLysLeuAlaAlaAlaLeuGluHisHisHisHisHisHisEnd
T7 terminator
T7 terminator primer #69337-3
TAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG



scFv-3T

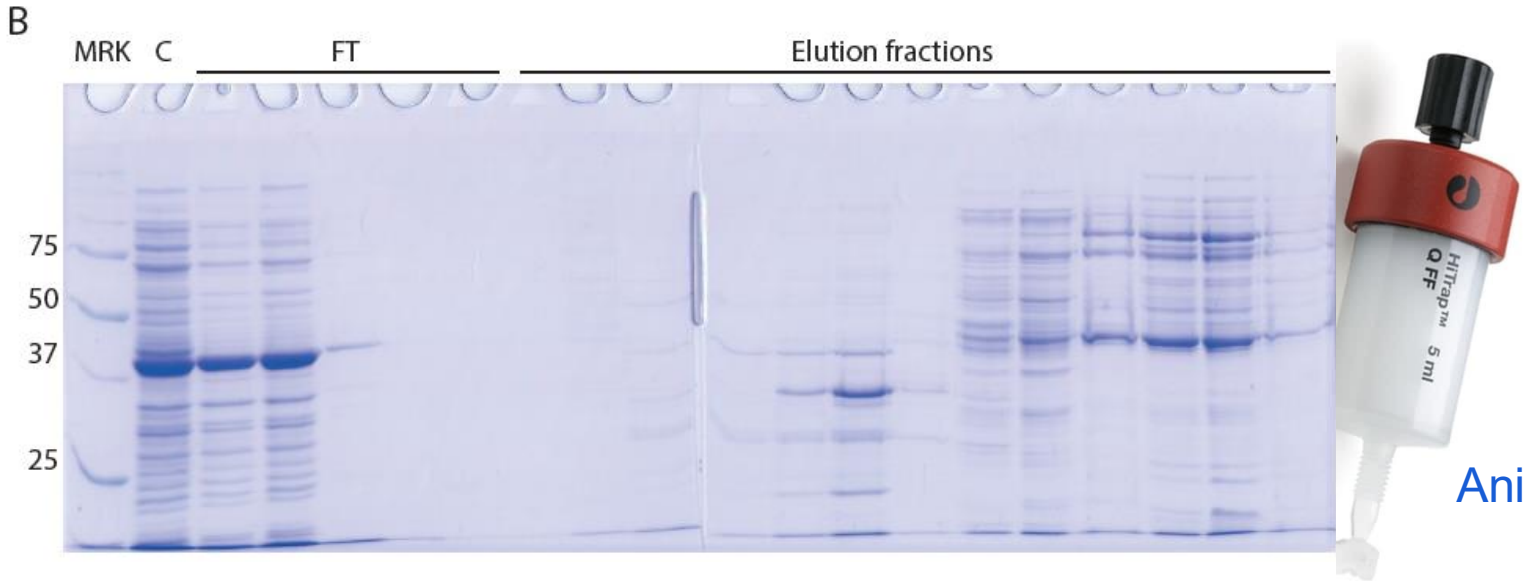

A
Soluble
IBs
MRK NI 1h 2h 3h NI 1h 2h 3h
75
50
37
25
B
Soluble
IBs
MRK NI 1h 2h 3h NI 1h 2h 3h
75
50
37
25


Abs 405 nm
4
3
2
1
0
UbDscFv
UbTscFv
50.000
25.000
12.500
6.250
3.125
1.560
0.780
0.390
0.195
0.098
scFvs concentrations (µg/ml)

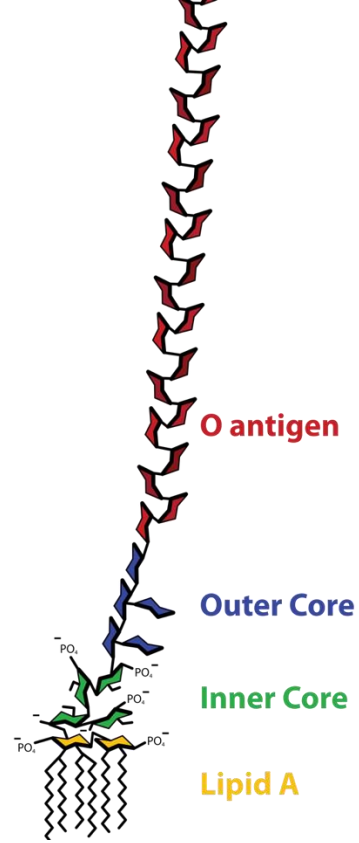

A
MRK C FT
Elution fractions
100 mM
Gragient to 250 mM
75
50
37
25

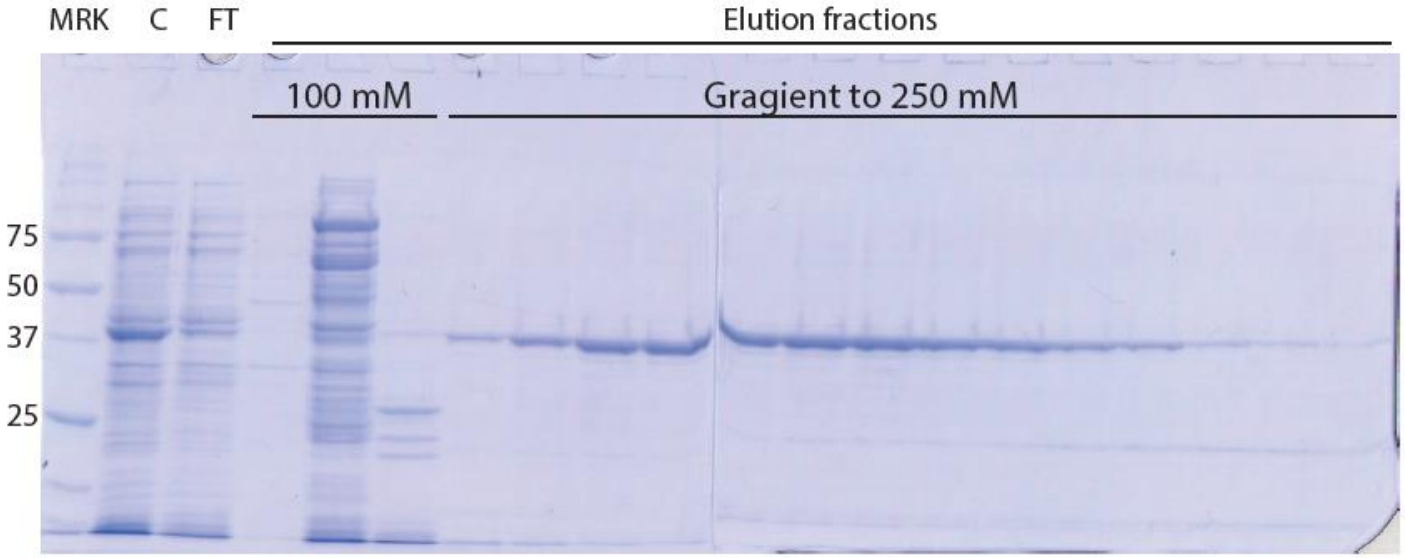

HisTrap™ HP
5 ml

scFv-3T



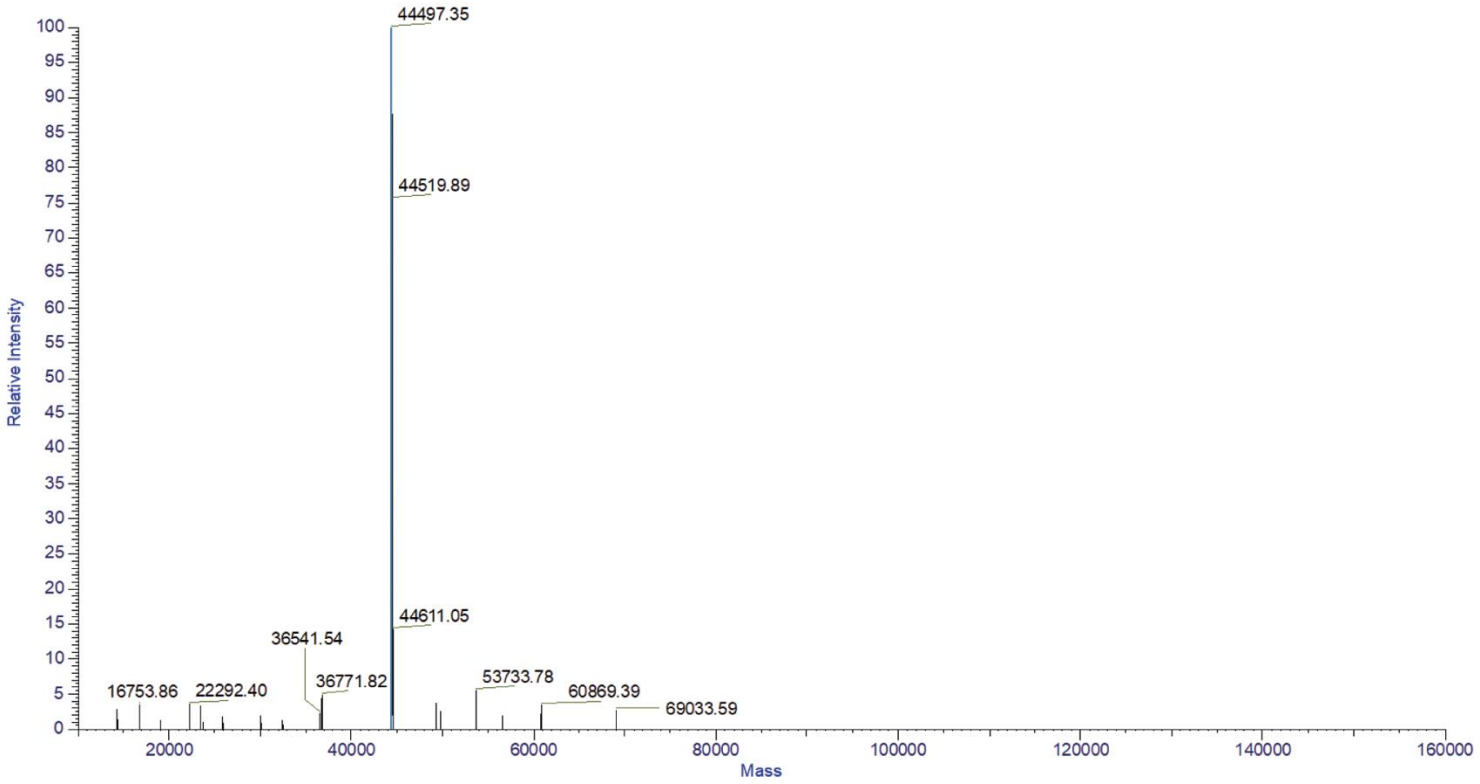
Anion exchange column

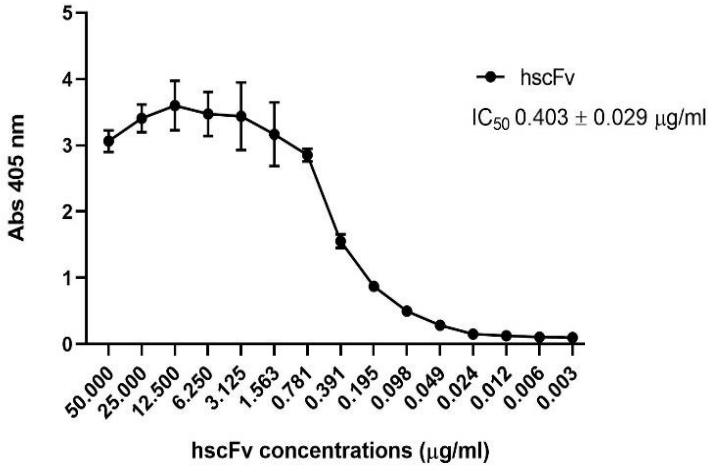




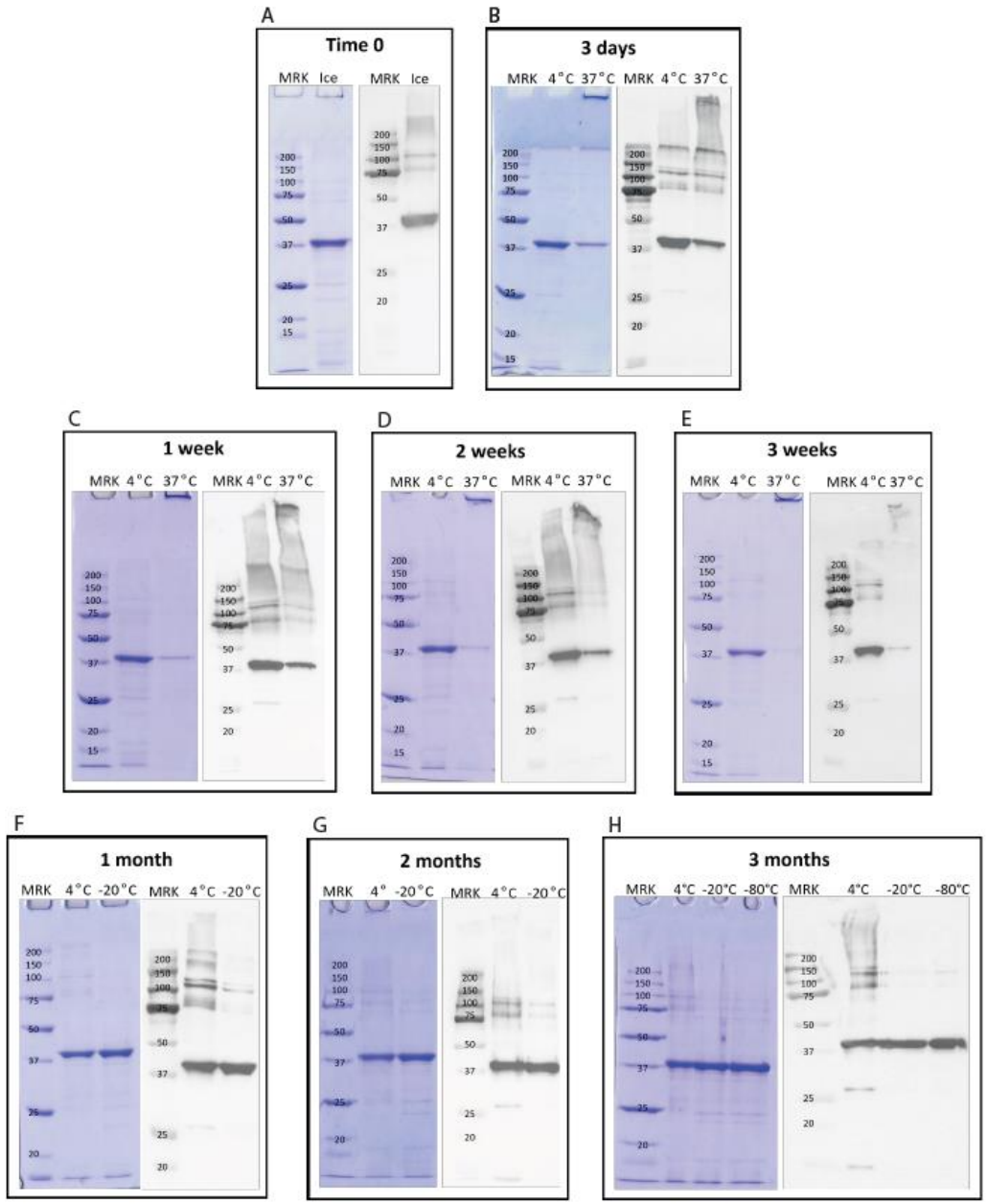
scFv-3T

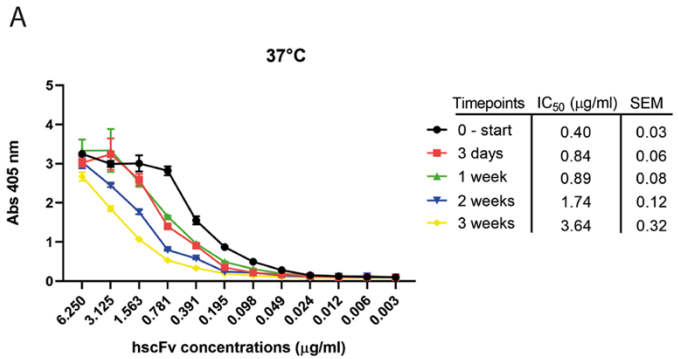
B

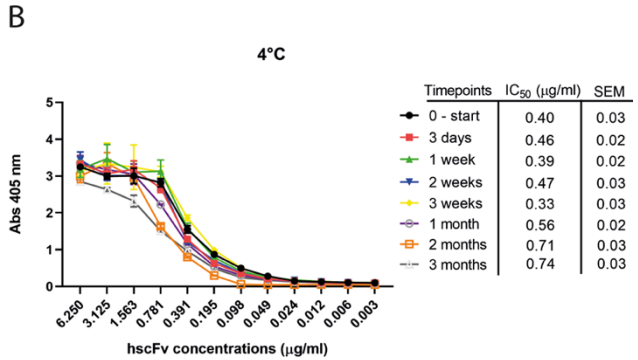

44497.35
44519.89
44611.05
36541.54
36771.82
16753.86
22292.40
53733.78
60869.39
69033.59
Relative Intensity
Mass
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
20000
40000
60000
80000
100000
120000
140000
160000

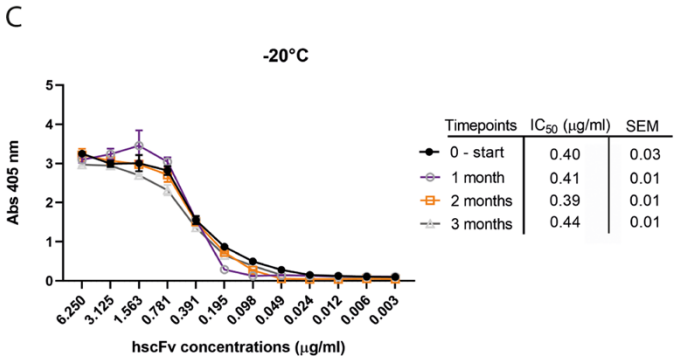

hscFv
IC50 0.403 ± 0.029 μg/ml
Abs 405 nm
5
4
3
2
1
0
50.000
25.000
12.500
6.250
3.125
1.563
0.781
0.391
0.195
0.098
0.049
0.024
0.012
0.006
0.003
hscFv concentrations (μg/ml)

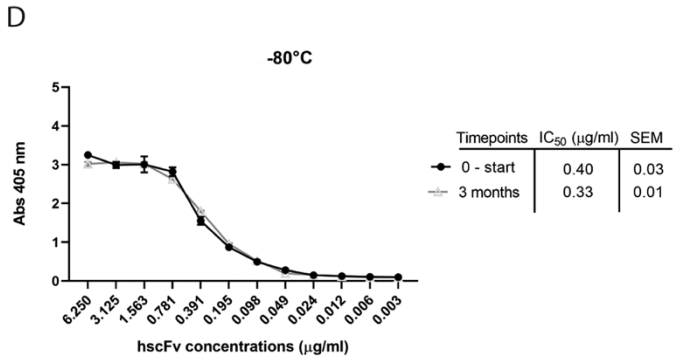
scFv-3T


A
Time 0
MRK Ice MRK Ice
B
3 days
MRK 4°C 37°C MRK 4°C 37°C
C
1 week
MRK 4°C 37°C MRK 4°C 37°C
D
2 weeks
MRK 4°C 37°C MRK 4°C 37°C
E
3 weeks
MRK 4°C 37°C MRK 4°C 37°C
F
1 month
MRK 4°C -20°C MRK 4°C -20°C
G
2 months
MRK 4° -20°C MRK 4°C -20°C
H
3 months
MRK 4°C -20°C -80°C MRK 4°C -20°C -80°C

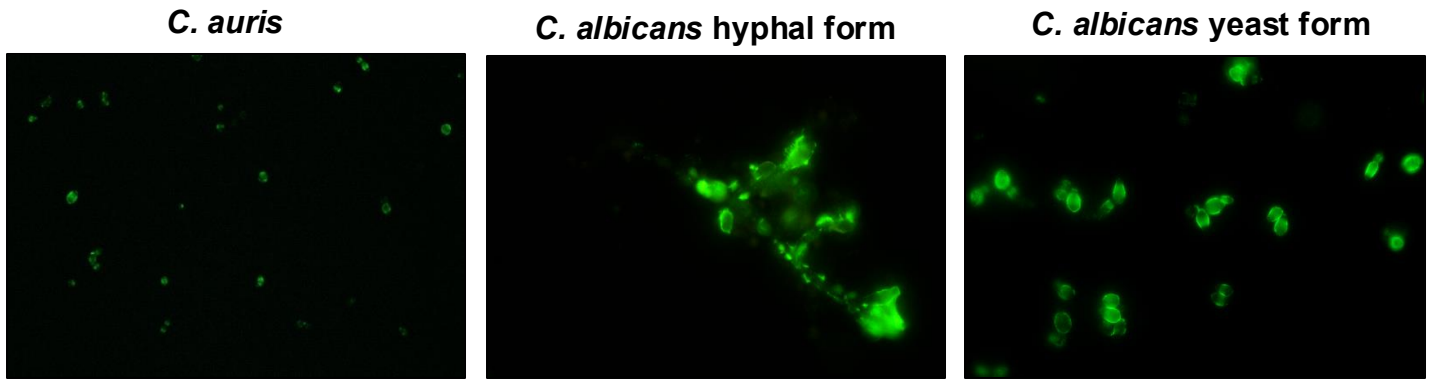

A
37°C
Abs 405 nm
hscFv concentrations (µg/ml)
6.250 3.125 1.563 0.781 0.391 0.195 0.098 0.049 0.024 0.012 0.006 0.003
Timepoints IC50 (µg/ml) SEM
0 - start 0.40 0.03
3 days 0.84 0.06
1 week 0.89 0.08
2 weeks 1.74 0.12
3 weeks 3.64 0.32

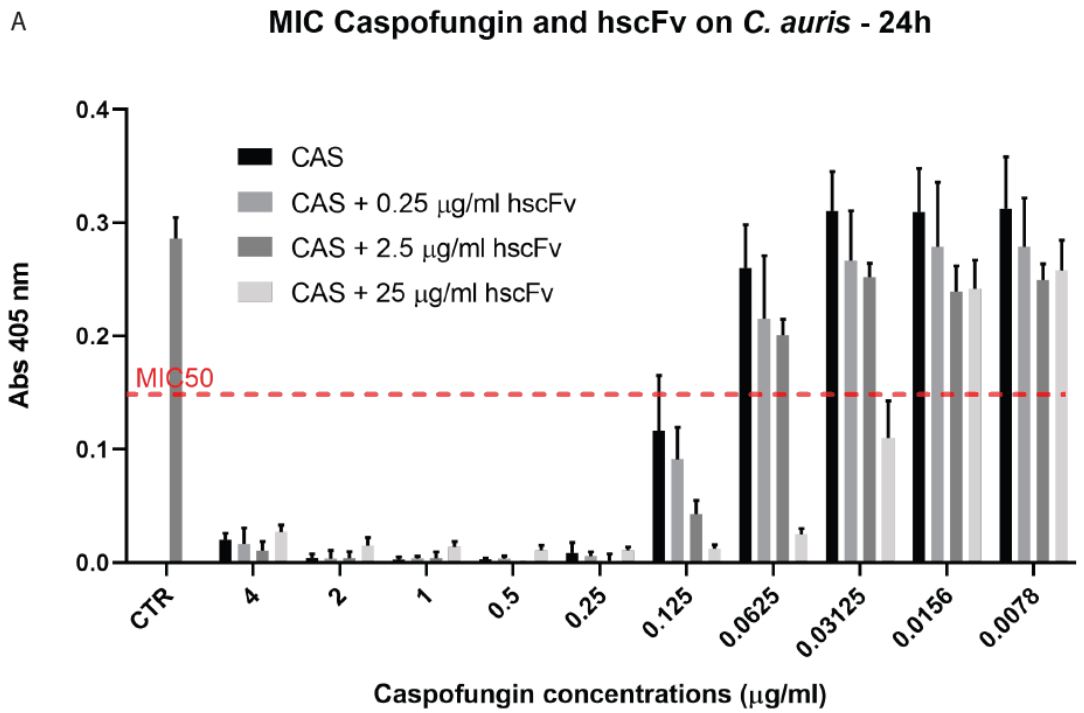

B
4°C
Abs 405 nm
hscFv concentrations (µg/ml)
6.250 3.125 1.563 0.781 0.391 0.195 0.098 0.049 0.024 0.012 0.006 0.003
Timepoints IC50 (µg/ml) SEM
0 - start 0.40 0.03
3 days 0.46 0.02
1 week 0.39 0.02
2 weeks 0.47 0.03
3 weeks 0.33 0.03
1 month 0.56 0.02
2 months 0.71 0.03
3 months 0.74 0.03

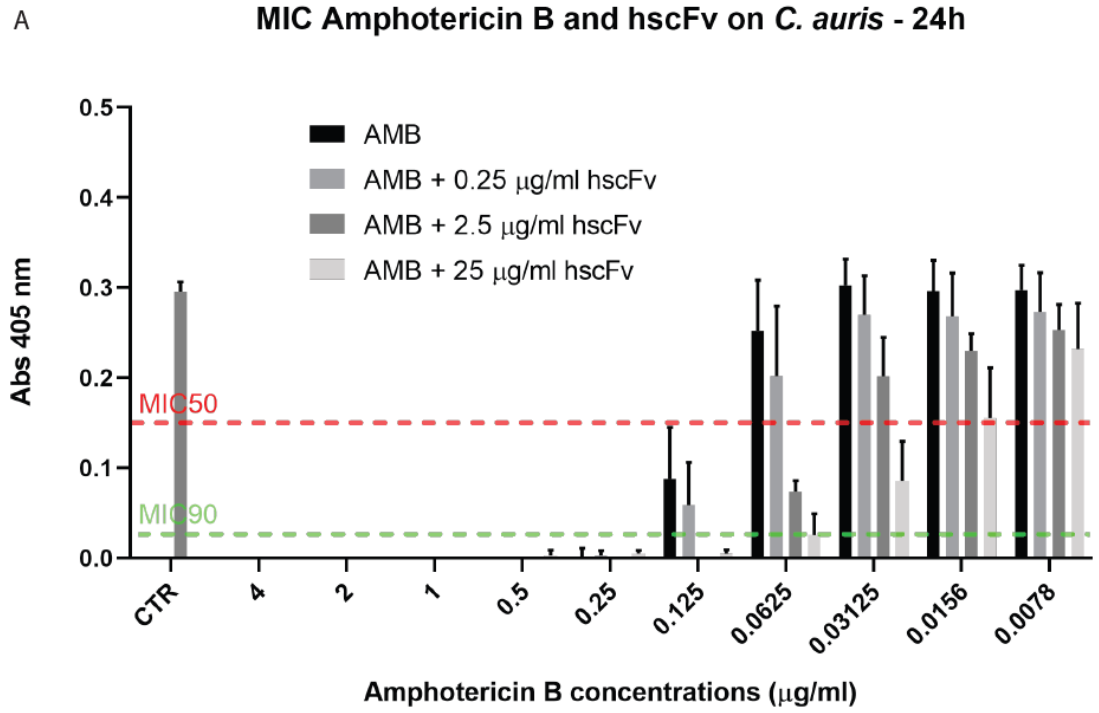

C
-20°C
Abs 405 nm
hscFv concentrations (µg/ml)
6.250 3.125 1.563 0.781 0.391 0.195 0.098 0.049 0.024 0.012 0.006 0.003
Timepoints IC50 (µg/ml) SEM
0 - start 0.40 0.03
1 month 0.41 0.01
2 months 0.39 0.01
3 months 0.44 0.01


D
-80°C
Abs 405 nm
hscFv concentrations (µg/ml)
6.250 3.125 1.563 0.781 0.391 0.195 0.098 0.049 0.024 0.012 0.006 0.003
Timepoints IC50 (µg/ml) SEM
0 - start 0.40 0.03
3 months 0.33 0.01

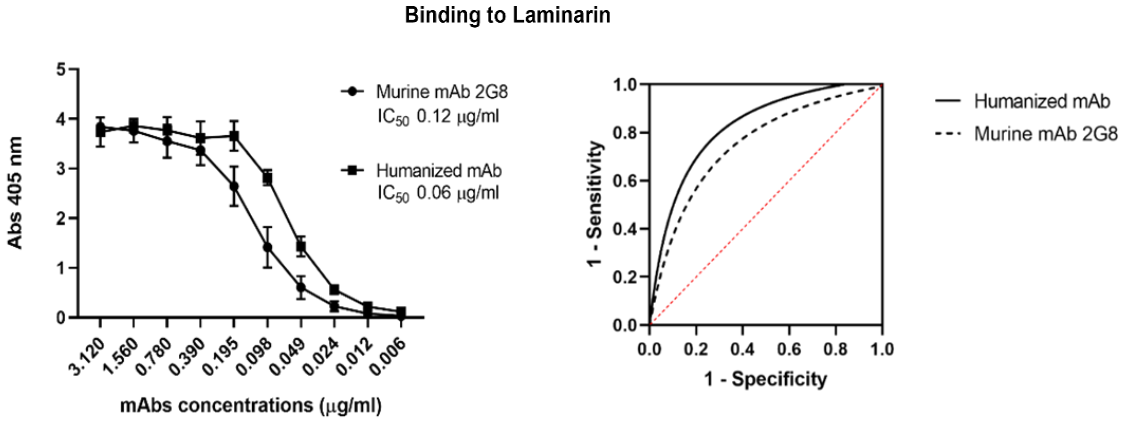
scFv-3T


C. auris
C. albicans hyphal form
C. albicans yeast form


A
MIC Caspofungin and hscFv on *C. auris* - 24h
Abs 405 nm
0.4
0.3
0.2
0.1
0.0
CAS
CAS + 0.25 µg/ml hscFv
CAS + 2.5 µg/ml hscFv
CAS + 25 µg/ml hscFv
MIC50
CTR
4
2
1
0.5
0.25
0.125
0.0625
0.03125
0.0156
0.0078
Caspofungin concentrations (µg/ml)

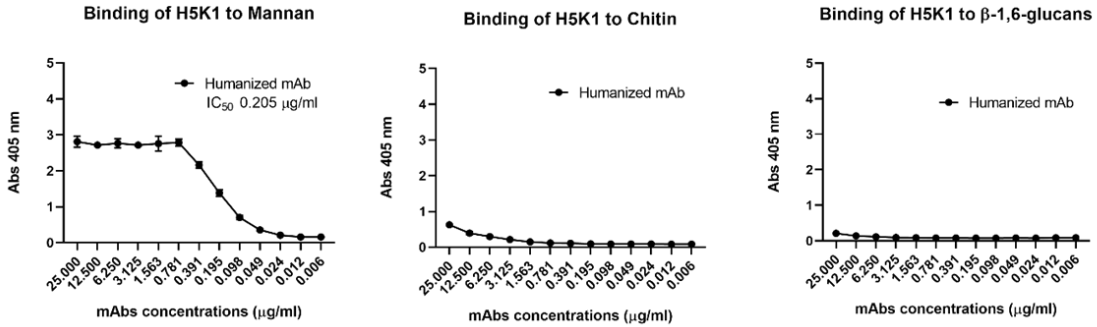

A
MIC Amphotericin B and hscFv on *C. auris* - 24h
Abs 405 nm
0.5
0.4
0.3
0.2
0.1
0.0
AMB
AMB + 0.25 µg/ml hscFv
AMB + 2.5 µg/ml hscFv
AMB + 25 µg/ml hscFv
MIC50
MIC90
CTR
4
2
1
0.5
0.25
0.125
0.0625
0.03125
0.0156
0.0078
Amphotericin B concentrations (µg/ml)

Dia-T51 binding *in vitro*

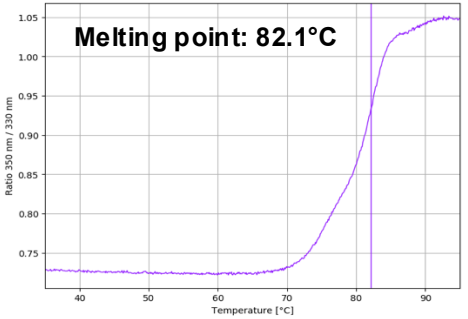


Murine-humanized mAb 2G8 comparison

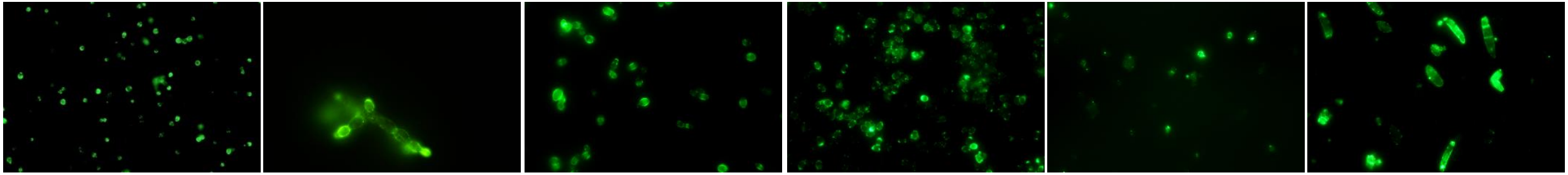
	IC_{50} (µg/ml)	AUC	K_d
Dia-T51	0.06	0.85	$4x10^{-10}$
2G8	0.12	0.77	$1.9x10^{-9}$



Stability



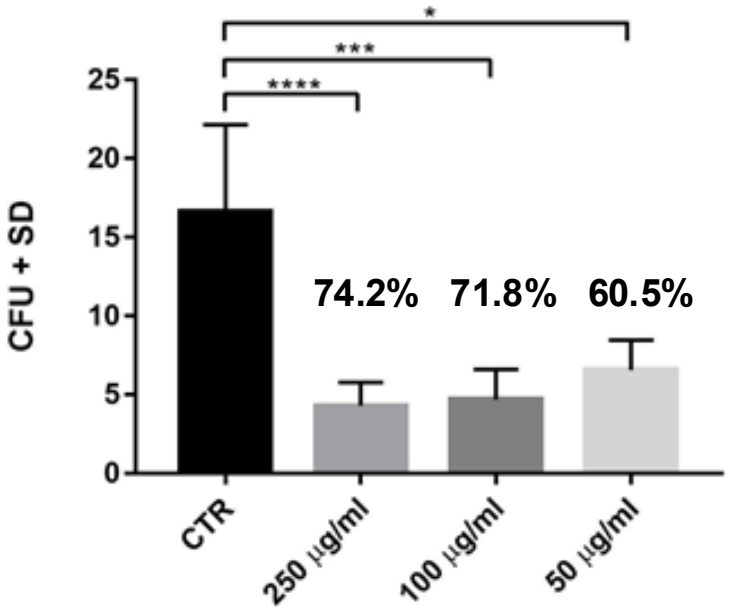
Dia-T51 IC_{50} (µg/ml)	6 months 4°C	12 months 4°C	18 months -80°C
	0.10	0.10	0.09



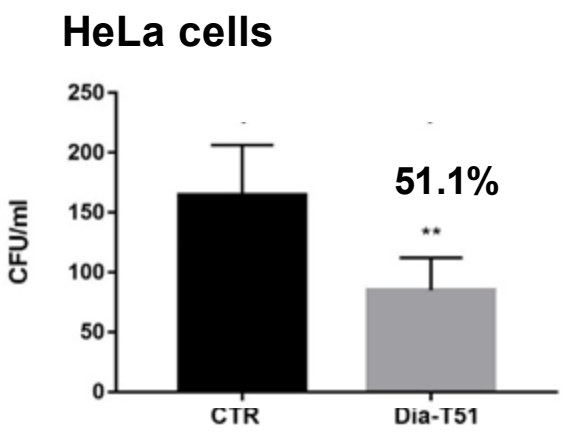
C. auris | *C. albicans* hyphal form | *C. albicans* yeast form | *C. glabrata* | *A. fumigatus* conidia | *F. solani* conidia

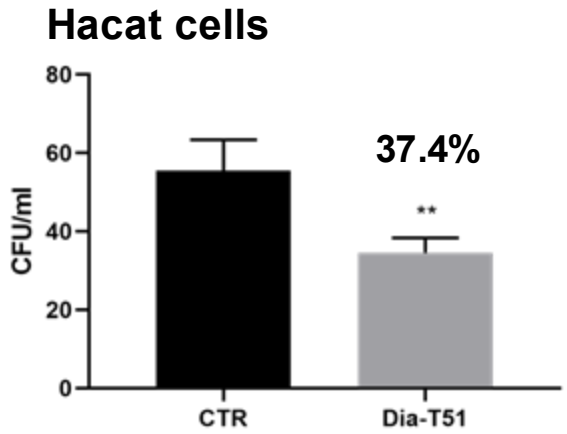
Dia-T51 effect alone

C. auris growth inhibition



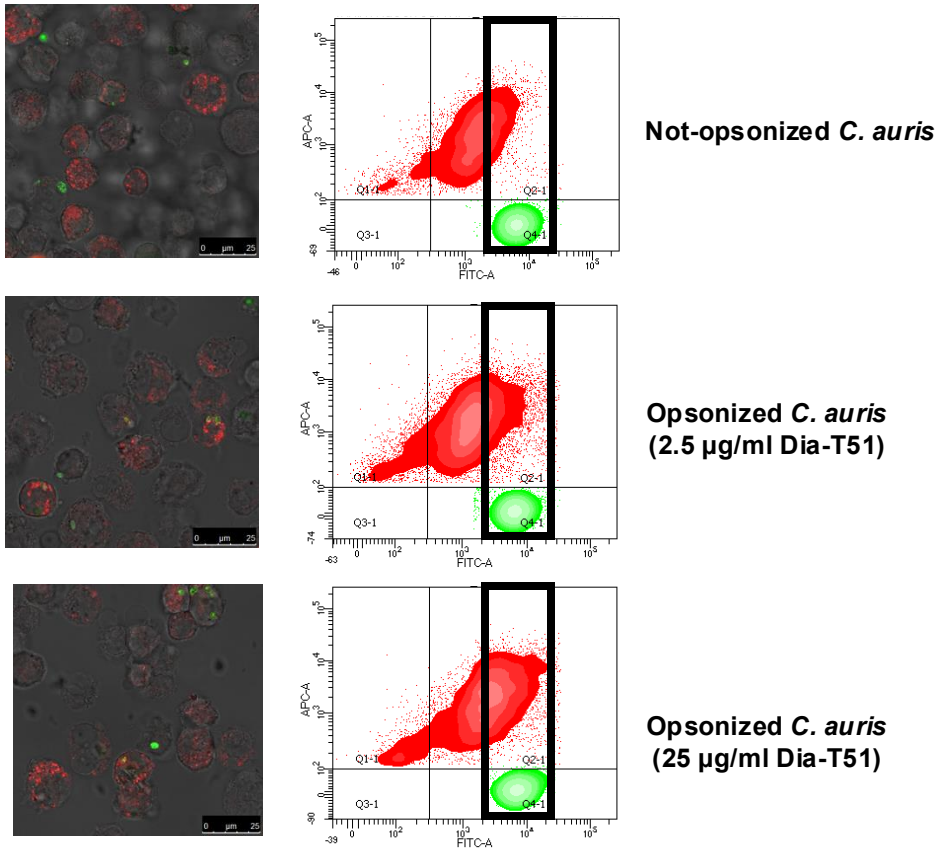
C. auris adhesion inhibition





Dia-T51 - phagocytosis

THP-1 cell line



Not-opsonized *C. auris*

Opsonized *C. auris* (2.5 µg/ml Dia-T51)

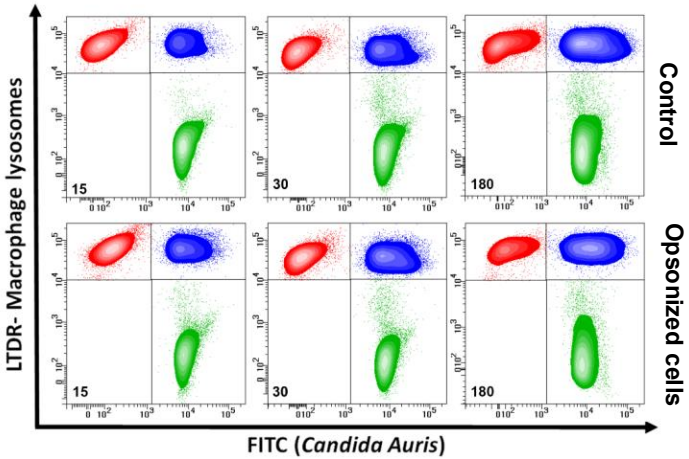
Opsonized *C. auris* (25 µg/ml Dia-T51)

Red population: THP-1 (human macrophages)
Green population: *C. auris*

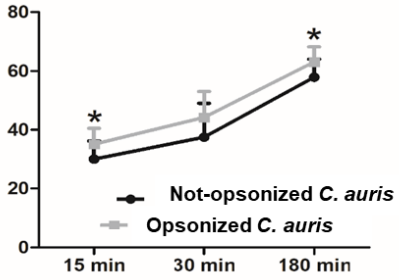
	Engulfing macrophages (%)	Residual Candida/Macrophages	Phagocytosis rate (%)
Not-opsonized *C. auris*	84.5	3.79	37.0
Opsonized *C. auris (2.5 µg/ml Dia-T51)*	85.8	2.44	46.5
Opsonized *C. auris (25 µg/ml Dia-T51)*	87.0	2.33	65.6

Human monocytes-derived macrophages

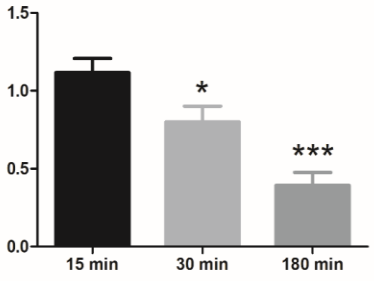
Red population: not-engulfing macrophages
Blue population: engulfing macrophages
Green population: residual *C. auris*



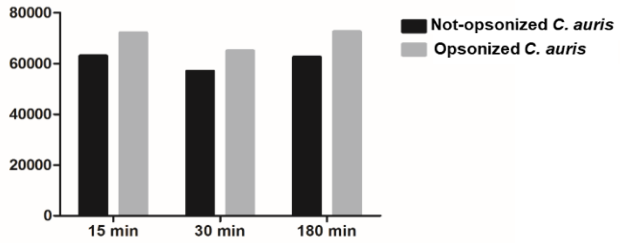
% phagocyting macrophages

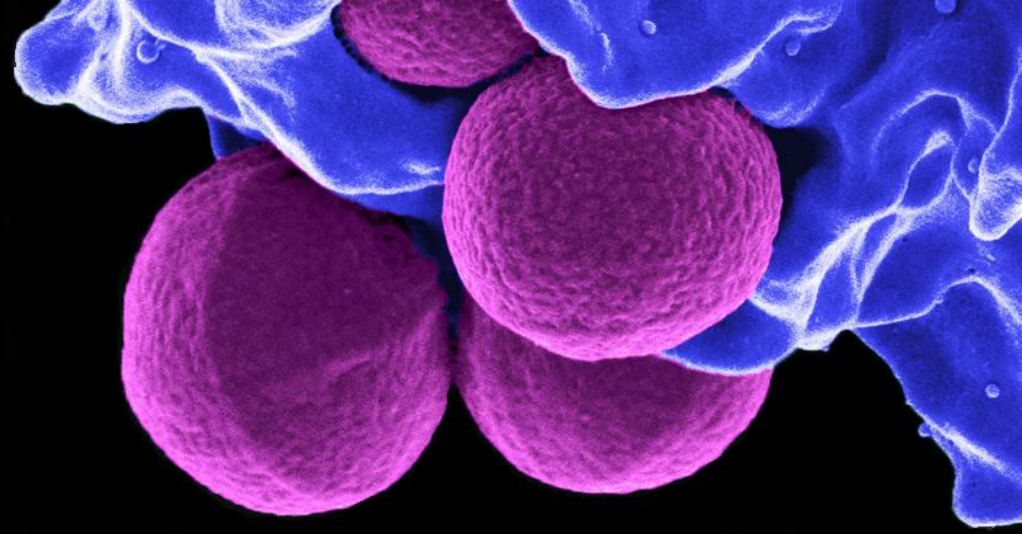


Fold of decrease of residual *C. auris*



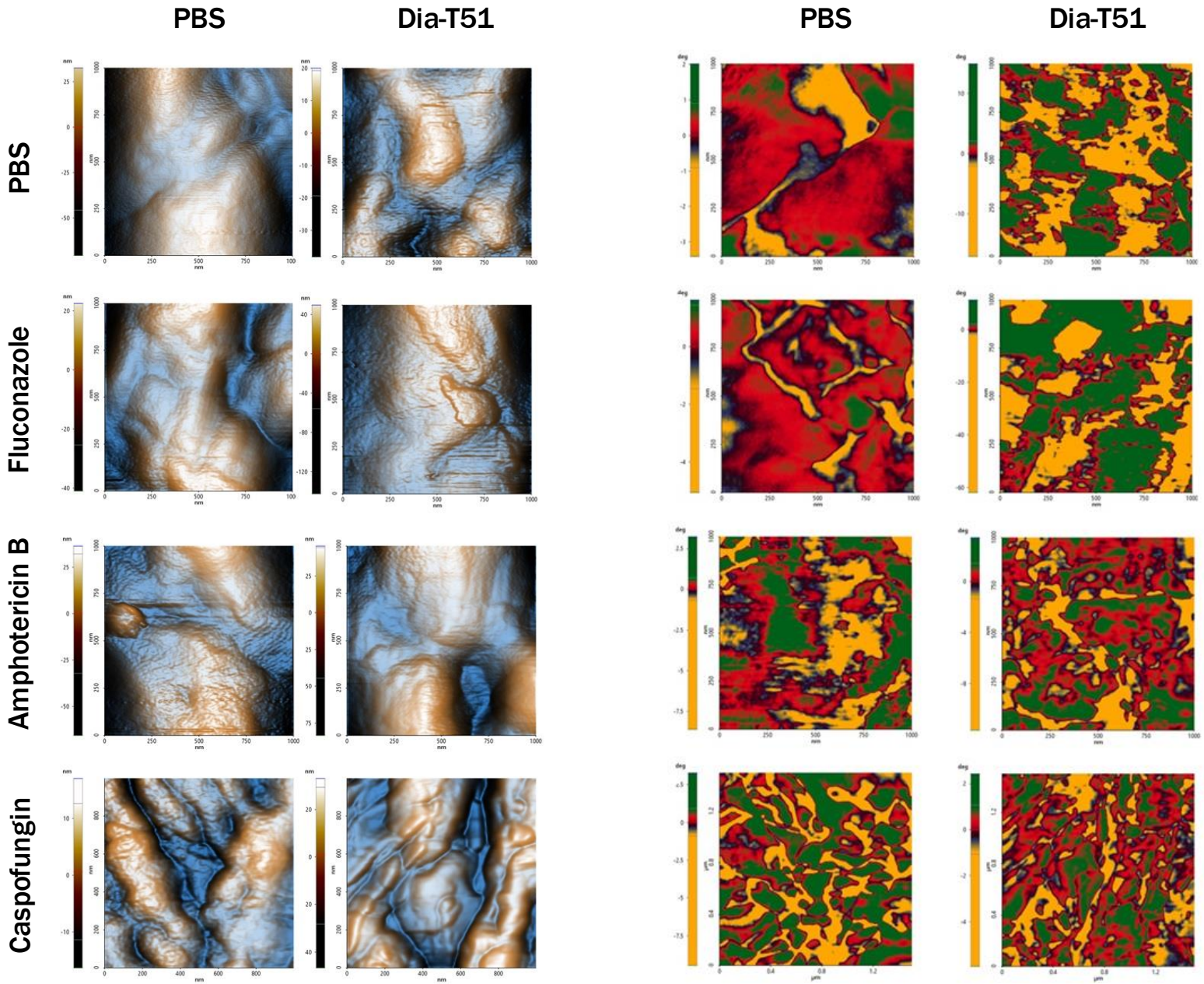
Lysosome activity





Antifungal drugs

FLUCONAZOLE	• **AZOLES** – inhibition of lanosterol 14α-demethylase and the subsequent conversion of lanosterol into ergosterol
CASPOFUNGIN	• **ECHINOCANDINS** – inhibition of β-1,3-glucan synthase and block of the β-1,3-glucan synthesis
AMPHOTERICIN B	• **POLYENES** – binding to ergosterol and formation of pores in the cell membrane



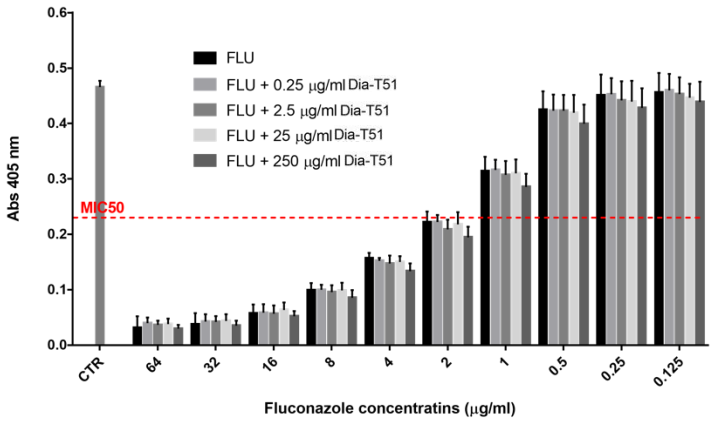
Atomic force microscopy

- Perturbation in the topographic and sub-topographic domains
- Higher frequency of vertical alterations
- Substantial difference in the chemical distribution
- Softening and weakening of the whole cell

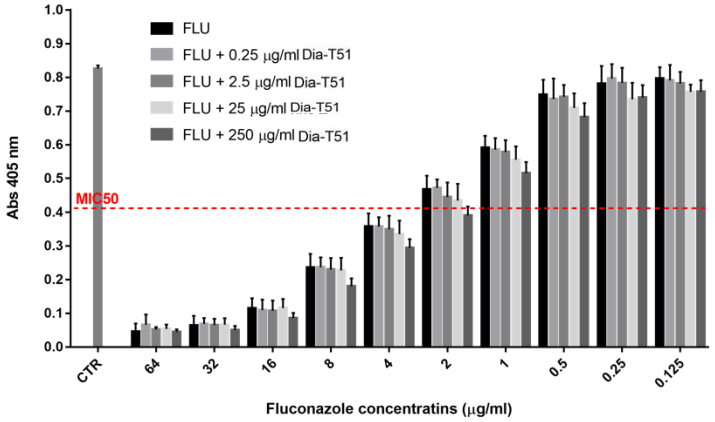
Dia-T51 effect in combination - *C. auris*

Dia-T51 + Fluconazole

24h

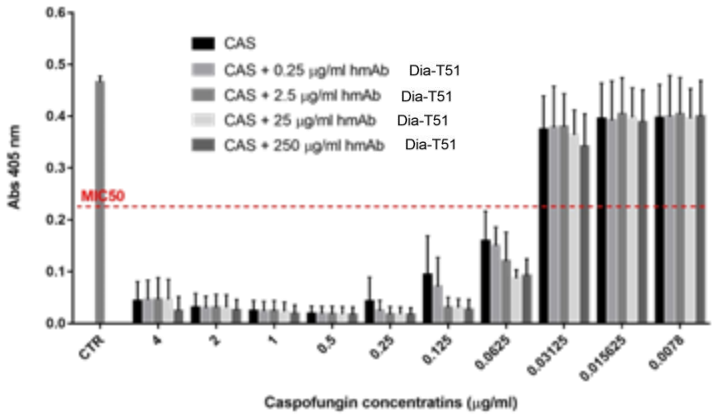


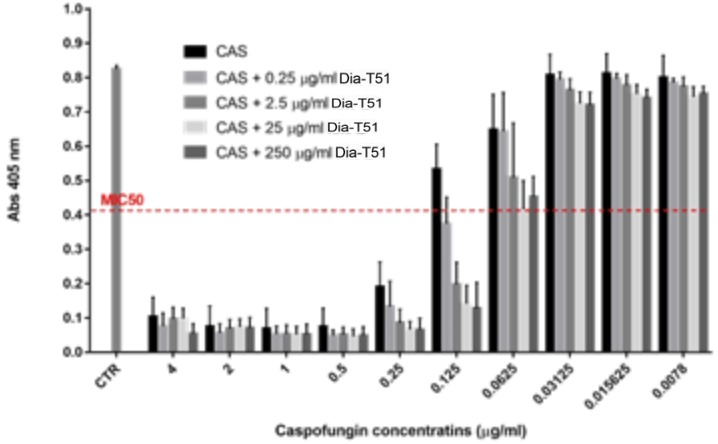
48h



Dia-T51 µg/ml	0	0.25	2.5	25	250
24h MIC FLU µg/ml	2	2	2	2	2
48h MIC FLU µg/ml	4	4	4	4	2

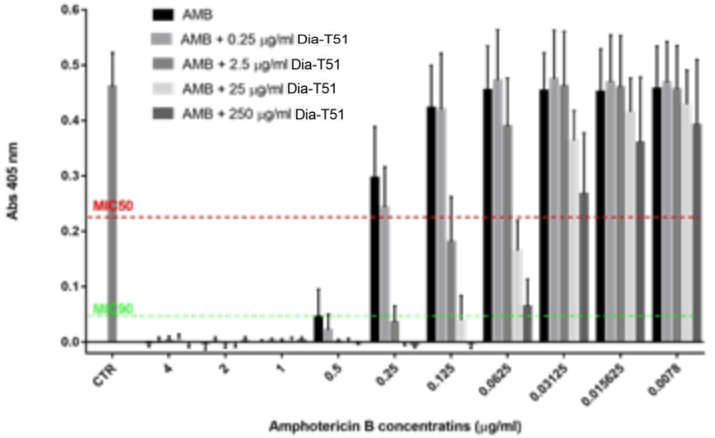
Dia-T51 + Caspofungin

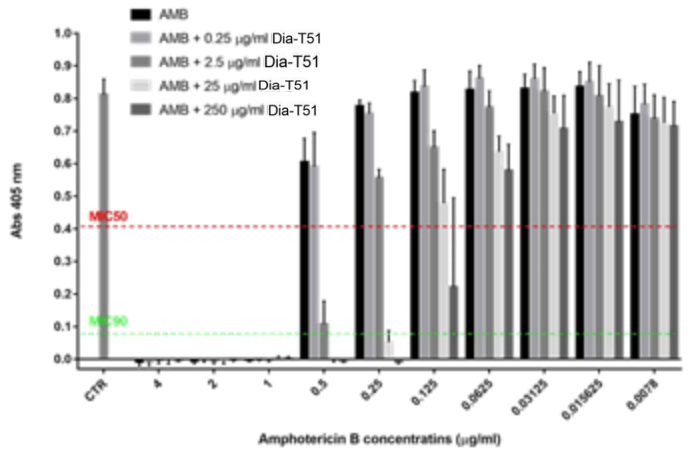




Dia-T51 µg/ml	0	0.25	2.5	25	250
24h MIC CAS µg/ml	0.0625	0.0625	0.0625	0.0625	0.0625
48h MIC CAS µg/ml	0.25	0.125	0.125	0.125	0.125

Dia-T51 + Amphotericin B

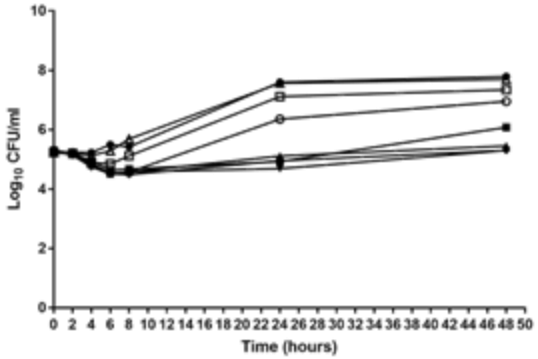




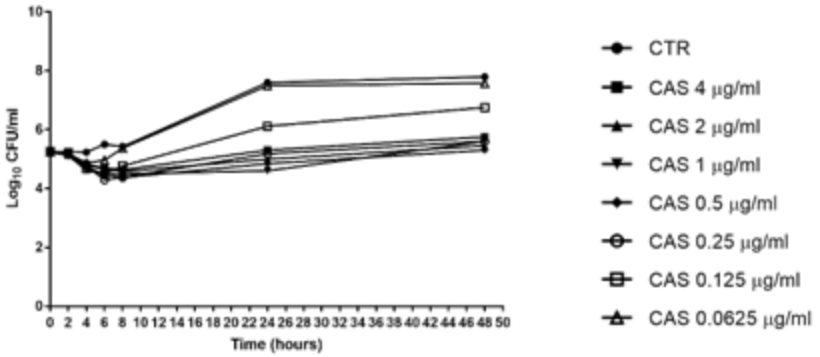
Dia-T51 µg/ml	0	0.25	2.5	25	250
24h MIC AMB µg/ml	0.5	0.5	0.25	0.125	0.125
48h MIC AMB µg/ml	1	1	1	0.25	0.25

Dia-T51 effect in combination - *C. auris*

Caspofungin



Caspofungin + 250 µg/ml Dia-T51

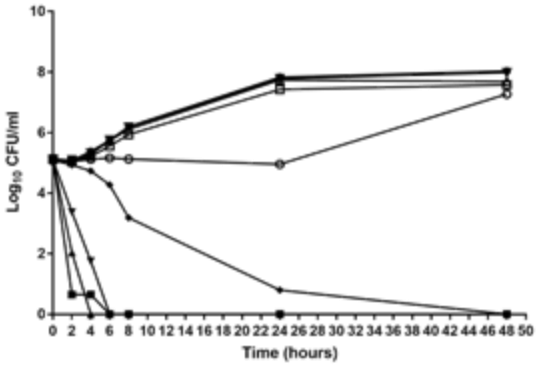


Fungistatic activity	24 h	48 h
CAS 0.25 µg/ml	-1.11	-1.71
CAS 0.25 µg/ml + 250 µg/ml Dia-T51	-0.02	-0.46
CAS 0.125 µg/ml	-1.88	-2.10
CAS 0.125 µg/ml + 250 µg/ml Dia-T51	-0.87	-1.51

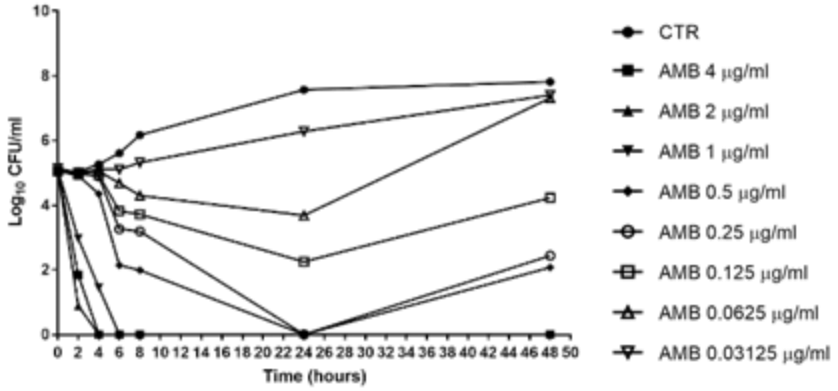
Δlog with the starting inoculum.

CAS and Dia-T51 are synergic per definition

Amphotericin B



Amphotericin B + 250 µg/ml Dia-T51



AMB concentrations	Δlog: AMB – AMB + 250 µg/ml Dia-T51	
	24 h	48 h
0.25 µg/ml	4.95	4.79
0.125 µg/ml	5.16	3.35
0.0625 µg/ml	4.04	0.4

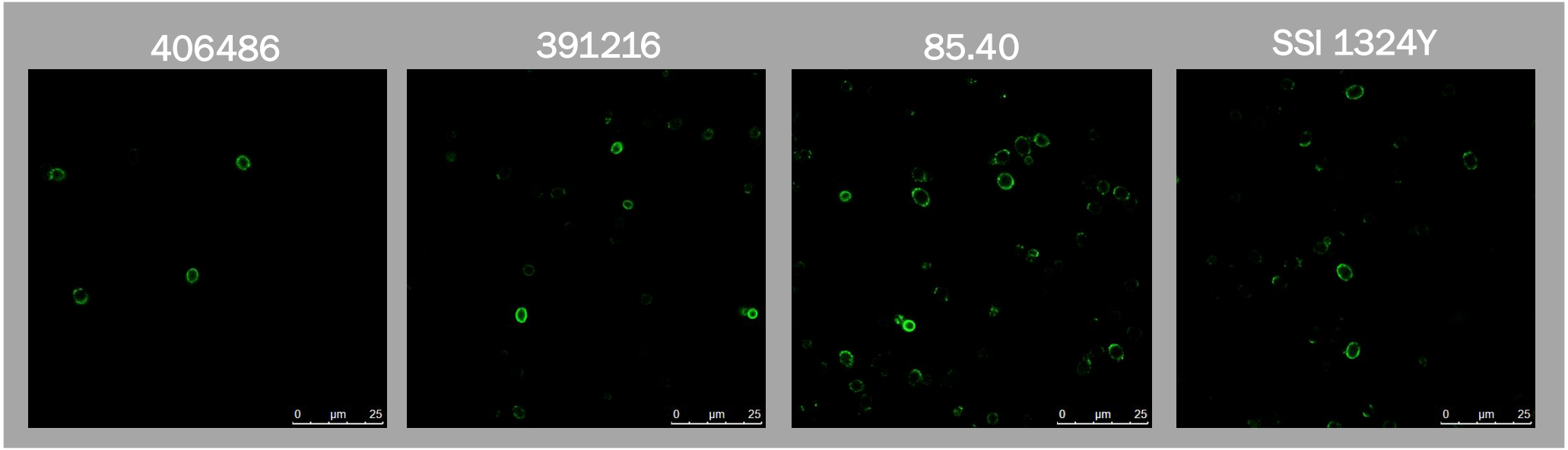
AMB and Dia-T51 are synergic

Fungicidal activity	0 h	2 h	4 h	6 h	8 h	24 h	48 h
AMB 4 µg/ml	0.06	≥3Log	≥3Log	≥3Log	≥3Log	≥3Log	≥3Log
AMB 4 µg+250 µg/ml Dia-T51	0.08	≥3Log	≥3Log	≥3Log	≥3Log	≥3Log	≥3Log
AMB 2 µg/ml	0.05	≥3Log	≥3Log	≥3Log	≥3Log	≥3Log	≥3Log
AMB 2 µg+250 µg/ml Dia-T51	0.10	≥3Log	≥3Log	≥3Log	≥3Log	≥3Log	≥3Log
AMB 1 µg/ml	0.05	1.77	≥3Log	≥3Log	≥3Log	≥3Log	≥3Log
AMB 1 µg+250 µg/ml Dia-T51	0.05	2.16	≥3Log	≥3Log	≥3Log	≥3Log	≥3Log
AMB 0.5 µg/ml	0.05	0.21	0.41	0.86	1.95	≥3Log	≥3Log
AMB 0.5 µg+250 µg/ml Dia-T51	0.09	0.22	0.78	≥3Log	≥3Log	≥3Log	≥3Log
AMB 0.25 µg/ml	0.07	0.10	0.02	-0.02	0.02	0.19	-2.13
AMB 0.25 µg+250 µg/ml Dia-T51	0.11	0.13	0.25	1.88	1.95	≥3Log	2.66
AMB 0.125 µg/ml	0.01	0.11	-0.07	-0.42	-.080	-2.28	-2.44
AMB 0.125 µg+250 µg/ml Dia-T51	0.09	0.20	0.21	1.31	1.42	2.88	0.91
AMB 0.0625 µg/ml	0	0.07	-0.14	-0.59	-0.97	-2.59	-2.57
AMB 0.0625 µg+250 µg/ml Dia-T51	0.09	0.15	0.09	0.46	0.84	1.45	-2.17
AMB 0.03125 µg/ml	0.01	0.05	-0.19	-0.62	-1.04	-2.65	-2.85
AMB 0.03125 µg+250 µg/ml Dia-T51	0.07	0.13	0.01	0.03	-0.18	-1.14	-2.26

- Is there synergy with other *Candida* spp.?
- Is it efficient with resistant strains?

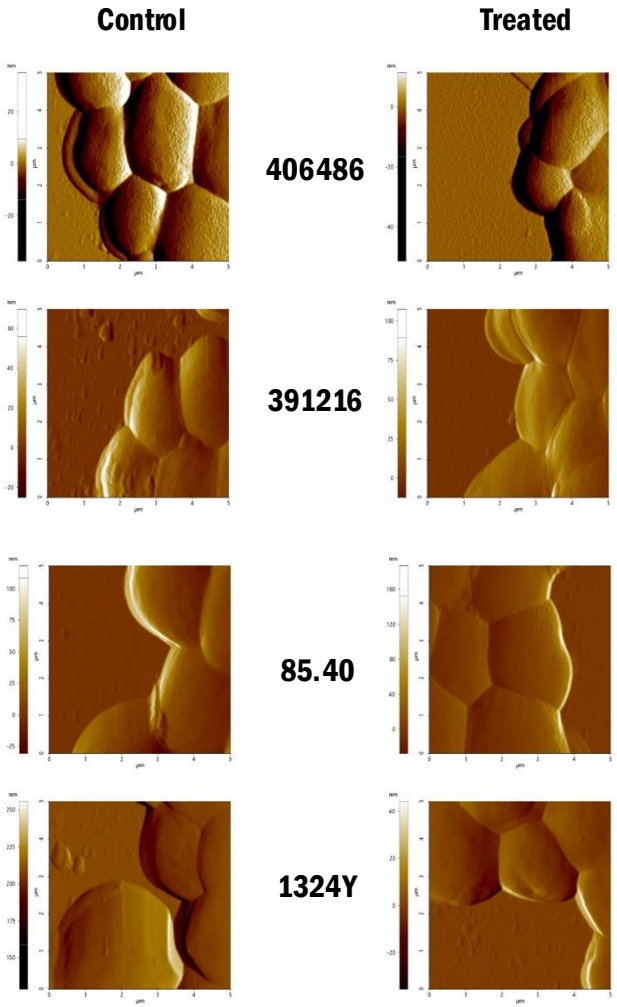
4 clinical isolates of *C. glabrata*

Dia-T51 effect in combination - *C. glabrata*

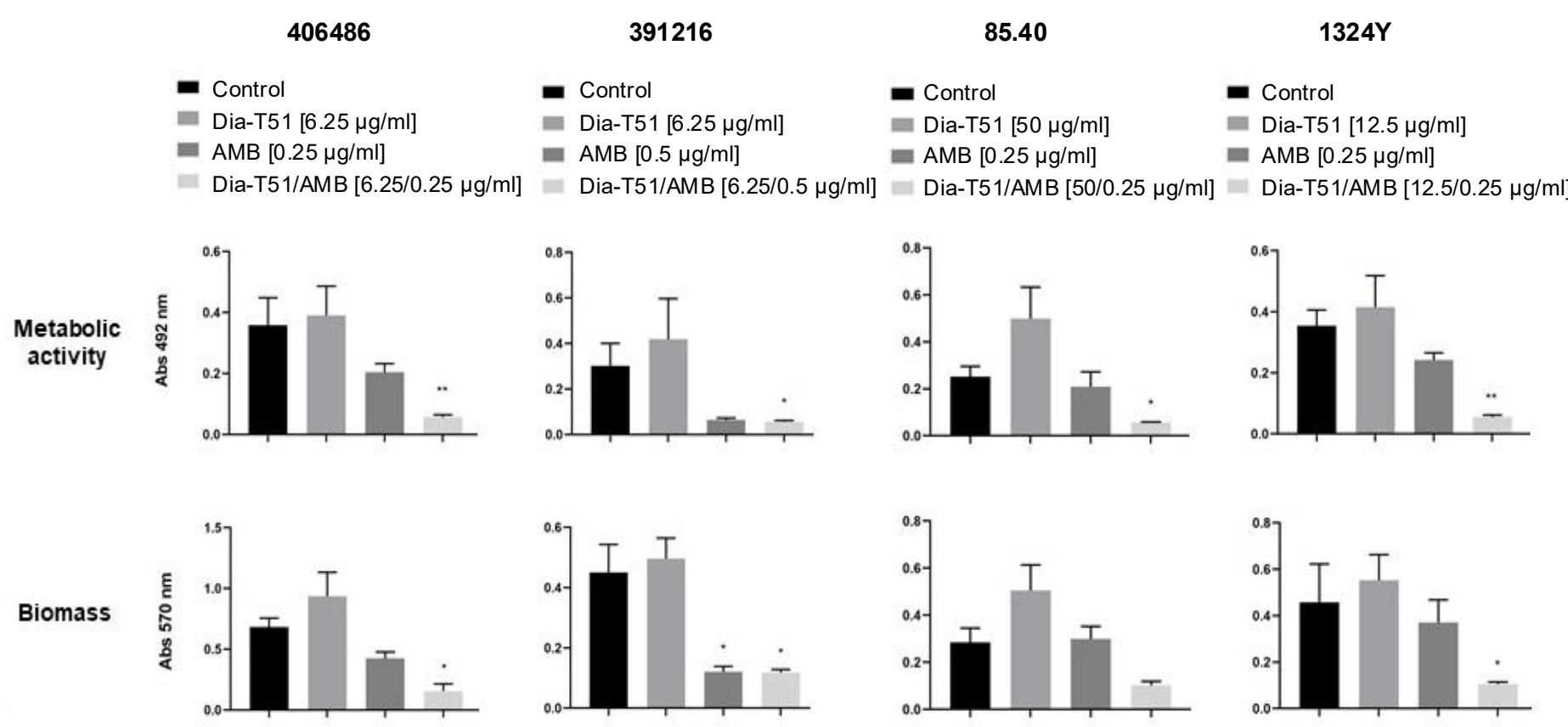


		Synergy in Checkerboard		Synergy in time-kill curves
***C. glabrata* strains**	**Characteristics**	**Dia-T51 / AMB (µg/ml)**	**FICI**	**Δlog most active compound vs combination ≥ 2 Dia-T51 / AMB (µg/ml)**
406486	Biofilm hyperproducer	0.78 / 0.125	0.252	6.25/0.25
391216	Common clinical isolate	1.56 / 0.25	0.254	6.25/0.5
85.40	Mutation in FKS gene	12.5 / 0.125	0.281	50/0.25
1324Y	Mutation in FKS gene	3.13 / 0.125	0.258	12.5/0.25

Dia-T51 effect in combination - *C. glabrata*



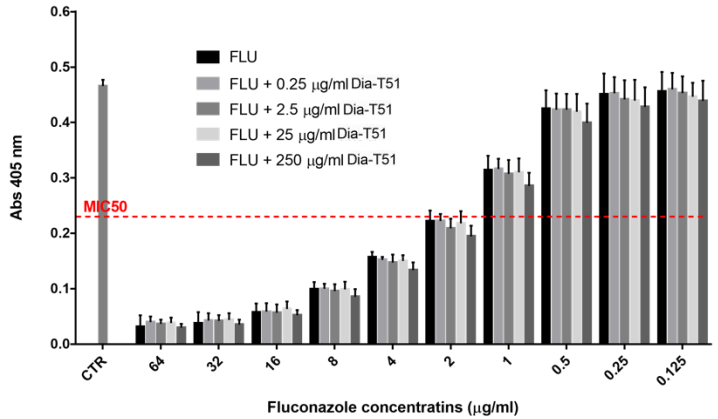
Anti-biofilm activity



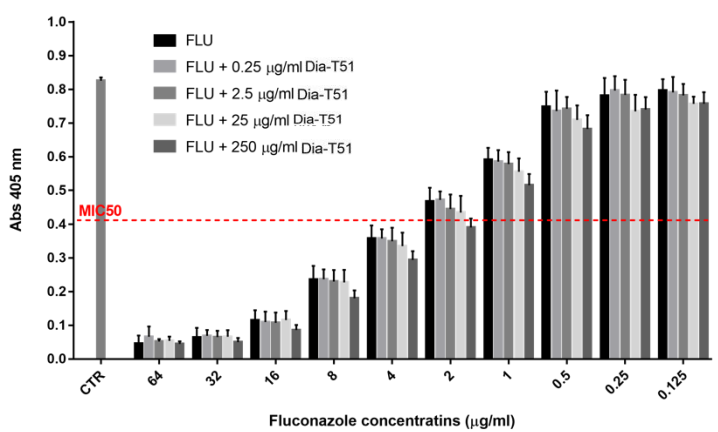
Dia-T51 effect in combination - *C. auris*

Dia-T51 + Fluconazole

24h

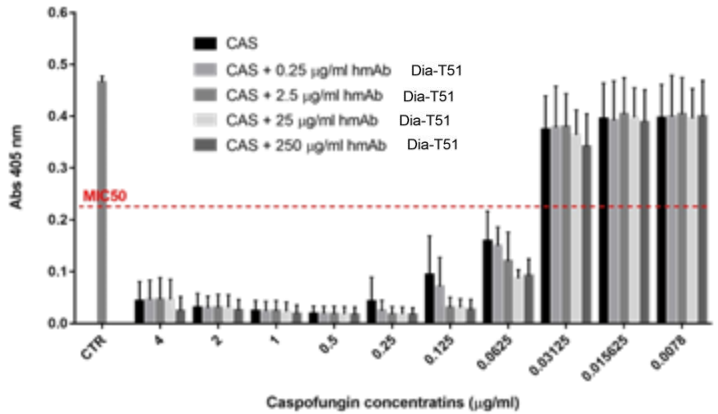


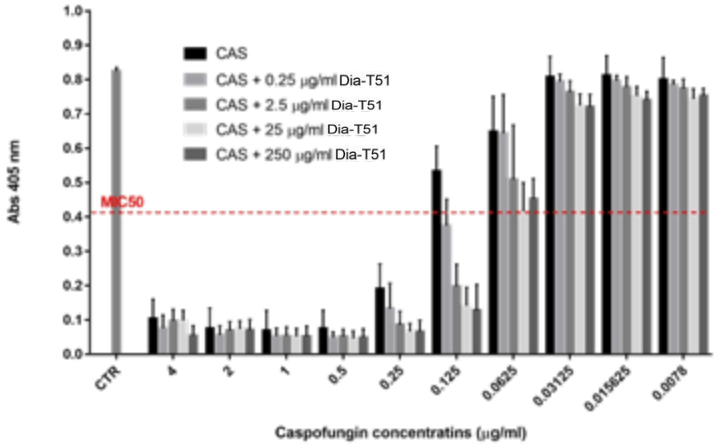
48h



Dia-T51 µg/ml	0	0.25	2.5	25	250
24h MIC FLU µg/ml	2	2	2	2	2
48h MIC FLU µg/ml	4	4	4	4	2

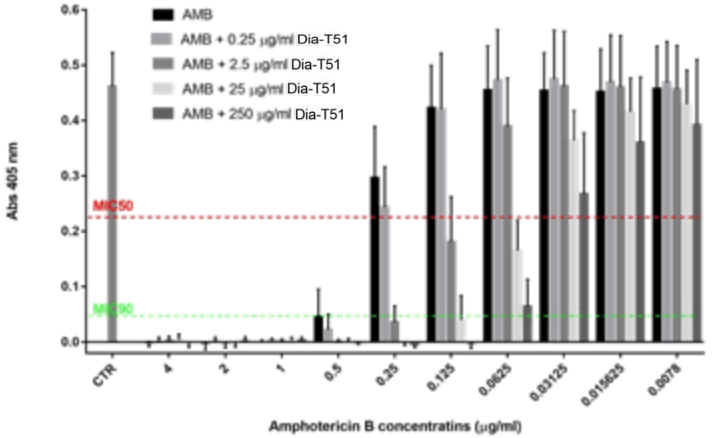
Dia-T51 + Caspofungin

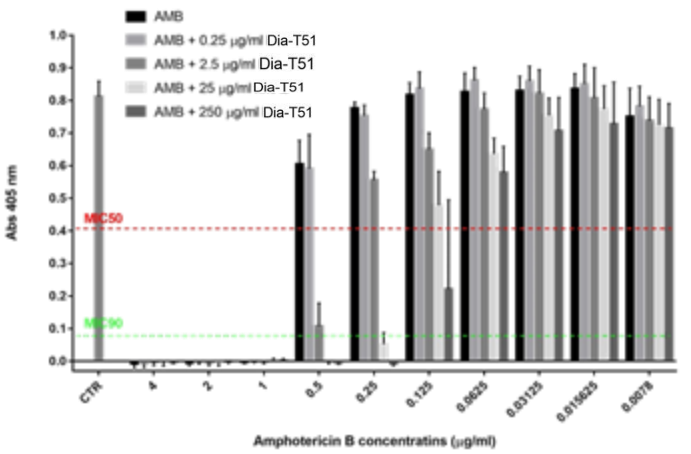




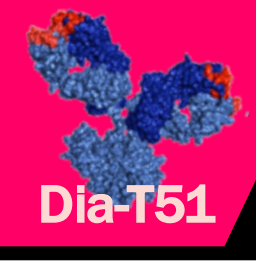
Dia-T51 µg/ml	0	0.25	2.5	25	250
24h MIC CAS µg/ml	0.0625	0.0625	0.0625	0.0625	0.0625
48h MIC CAS µg/ml	0.25	0.125	0.125	0.125	0.125

Dia-T51 + Amphotericin B



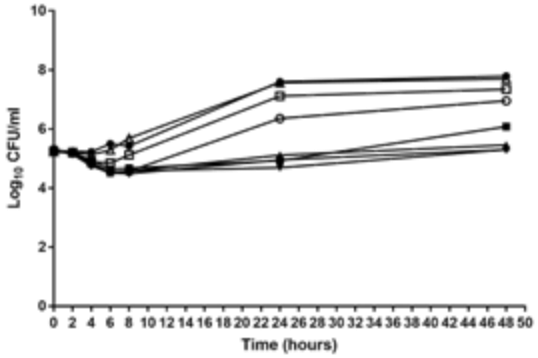


Dia-T51 µg/ml	0	0.25	2.5	25	250
24h MIC AMB µg/ml	0.5	0.5	0.25	0.125	0.125
48h MIC AMB µg/ml	1	1	1	0.25	0.25

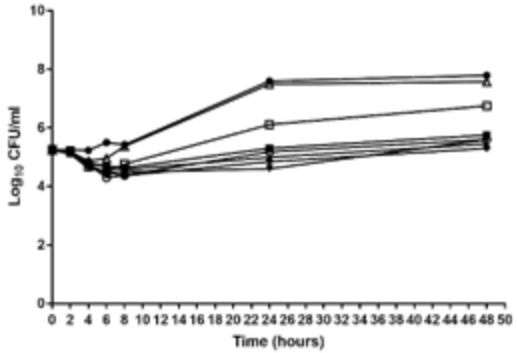


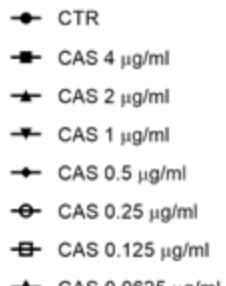
Dia-T51 effect in combination - *C. auris*

Caspofungin



Caspofungin + 250 µg/ml Dia-T51



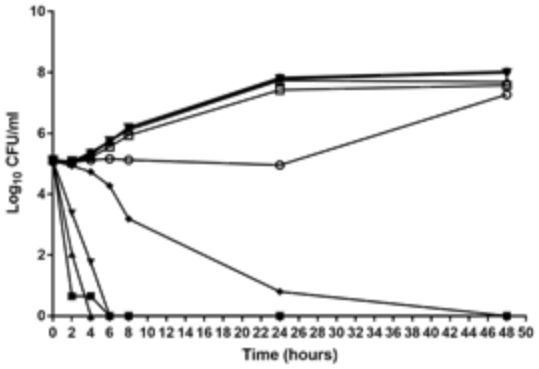


Fungistatic activity	24 h	48 h
CAS 0.25 µg/ml	-1.11	-1.71
CAS 0.25 µg/ml + 250 µg/ml Dia-T51	-0.02	-0.46
CAS 0.125 µg/ml	-1.88	-2.10
CAS 0.125 µg/ml + 250 µg/ml Dia-T51	-0.87	-1.51

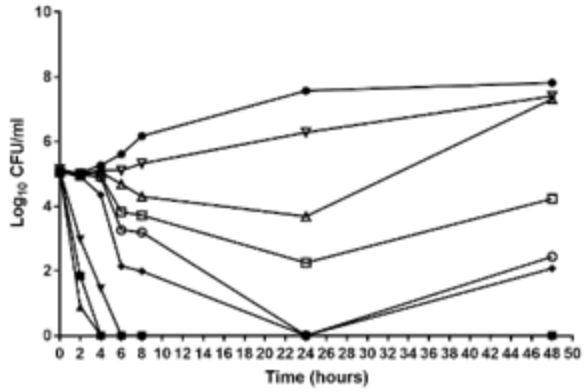
Δlog with the starting inoculum.

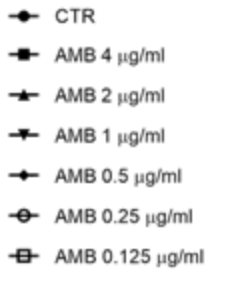
CAS and Dia-T51 are synergic per definition

Amphotericin B



Amphotericin B + 250 µg/ml Dia-T51





AMB concentrations	Δlog: AMB – AMB + 250 µg/ml Dia-T51	
	24 h	48 h
0.25 µg/ml	4.95	4.79
0.125 µg/ml	5.16	3.35
0.0625 µg/ml	4.04	0.4

AMB and Dia-T51 are synergic

Fungicidal activity	0 h	2 h	4 h	6 h	8 h	24 h	48 h
AMB 4 µg/ml	0.06	≥3Log	≥3Log	≥3Log	≥3Log	≥3Log	≥3Log
AMB 4 µg+250 µg/ml Dia-T51	0.08	≥3Log	≥3Log	≥3Log	≥3Log	≥3Log	≥3Log
AMB 2 µg/ml	0.05	≥3Log	≥3Log	≥3Log	≥3Log	≥3Log	≥3Log
AMB 2 µg+250 µg/ml Dia-T51	0.10	≥3Log	≥3Log	≥3Log	≥3Log	≥3Log	≥3Log
AMB 1 µg/ml	0.05	1.77	≥3Log	≥3Log	≥3Log	≥3Log	≥3Log
AMB 1 µg+250 µg/ml Dia-T51	0.05	2.16	≥3Log	≥3Log	≥3Log	≥3Log	≥3Log
AMB 0.5 µg/ml	0.05	0.21	0.41	0.86	1.95	≥3Log	≥3Log
AMB 0.5 µg+250 µg/ml Dia-T51	0.09	0.22	0.78	≥3Log	≥3Log	≥3Log	≥3Log
AMB 0.25 µg/ml	0.07	0.10	0.02	-0.02	0.02	0.19	-2.13
AMB 0.25 µg+250 µg/ml Dia-T51	0.11	0.13	0.25	1.88	1.95	≥3Log	2.66
AMB 0.125 µg/ml	0.01	0.11	-0.07	-0.42	-.080	-2.28	-2.44
AMB 0.125 µg+250 µg/ml Dia-T51	0.09	0.20	0.21	1.31	1.42	2.88	0.91
AMB 0.0625 µg/ml	0	0.07	-0.14	-0.59	-0.97	-2.59	-2.57
AMB 0.0625 µg+250 µg/ml Dia-T51	0.09	0.15	0.09	0.46	0.84	1.45	-2.17
AMB 0.03125 µg/ml	0.01	0.05	-0.19	-0.62	-1.04	-2.65	-2.85
AMB 0.03125 µg+250 µg/ml Dia-T51	0.07	0.13	0.01	0.03	-0.18	-1.14	-2.26