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High cell density cultivations for influenza virus production using suspension cells

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Outline

- Introduction: Designer cells for influenza vaccine production

- Results:

  Low Cell Density
  - CAP cells in serum free medium (SFM)
  - CAP cells in chemically defined medium (CDM)

  High Cell Density
  - CAP cells in CDM
  - AGE1.CR cells in CDM

- Summary

Host cell lines for influenza

cell line	origin	tissue	immortalization	"issues"	used by
MDCK	dog	kidney	spontaneous transformation	only low passage #	Medimmune Novartis
MDBK	bovine	kidney	spontaneous transformation	only low passage #	IDT Biologika
Vero	monkey	kidney	spontaneous transformation	only low passage # multilayer	Baxter
PER.C6	human	embryonic retinoblasts	transformation with adenovirus functions	ethical concerns? difficult for research	Crucell
HEK293	human	embryonic kidney	transformation with adenovirus functions	ethical concerns?	NRC Canada (A. Kamen; P17)
CAP	human	amniocytes	transformation with adenovirus functions	new	?
AGE1.CR **AGE1.CR.pIX**	duck	retinoblasts	transformation with adenovirus functions	relatively new	Ceva Santé Animale
EB66	duck	embryonic stem cells	no transformation, not modified	no peer-reviewed data difficult for research	GSK

Genzel Y. , Reichl U. *Exp. Rev. Vacc.* 2009;8(12):1681-92.
Jordan I. et al. *Vaccine.* 2009; 27(5):748-56.
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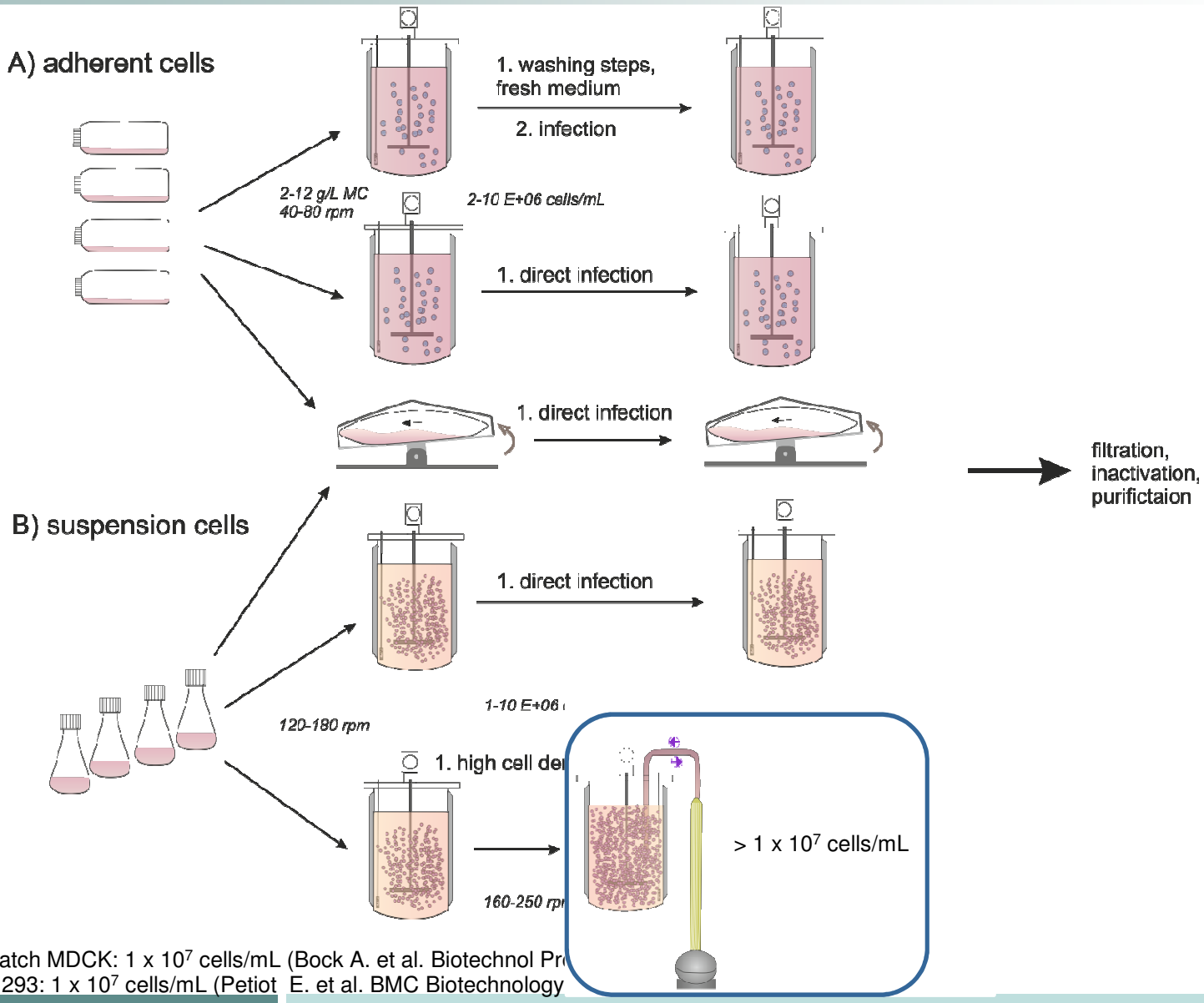
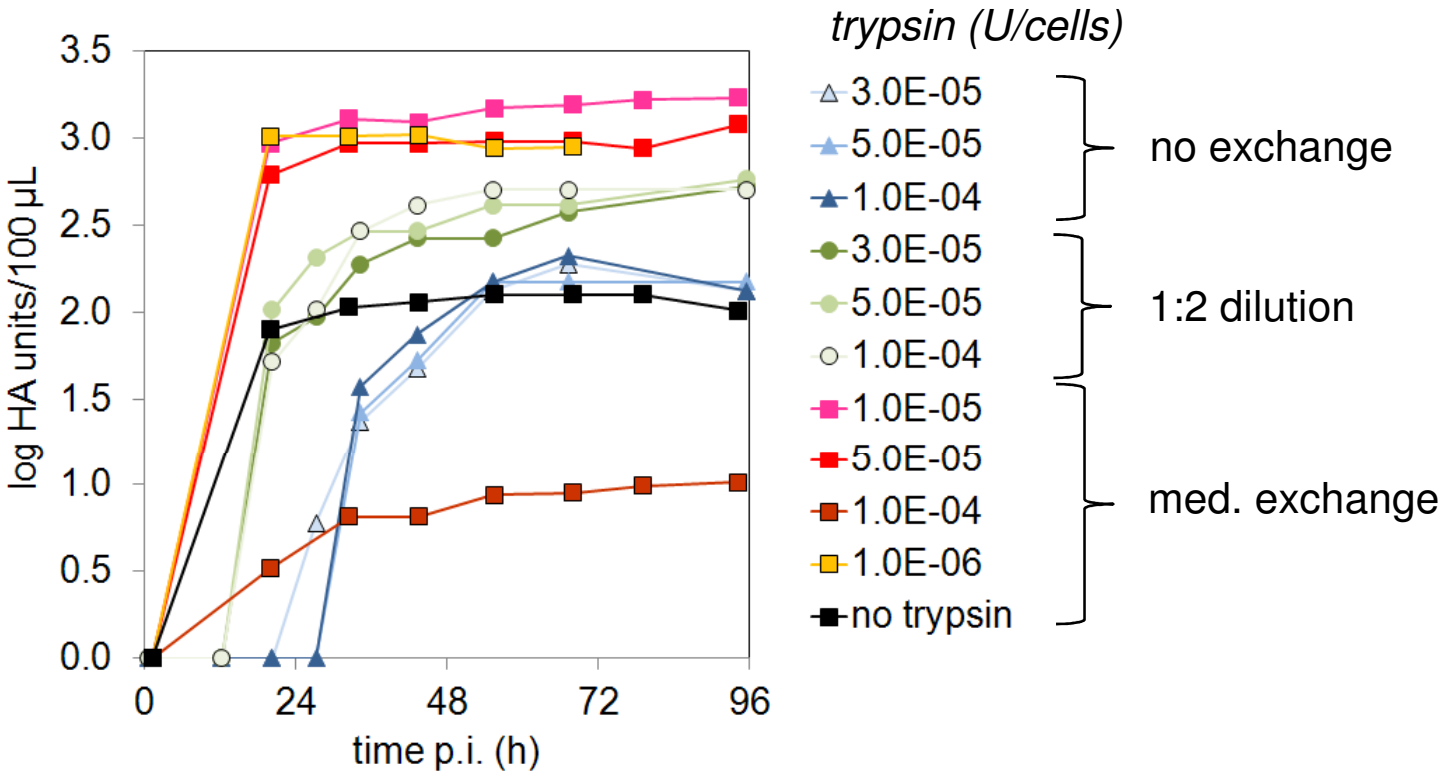
Paillet C. et al. *Vaccine*. 2009; 27: 6464-7.
Genzel Y. et al. *Appl. Microbiol. Biotechnol.* 2010; 88: 461-75.

Cell culture-based influenza production ...



perfusion/fed batch MDCK: 1 x 10^7 cells/mL (Bock A. et al. Biotechnol Pr
perfusion HEK.293: 1 x 10^7 cells/mL (Petiot E. et al. BMC Biotechnology

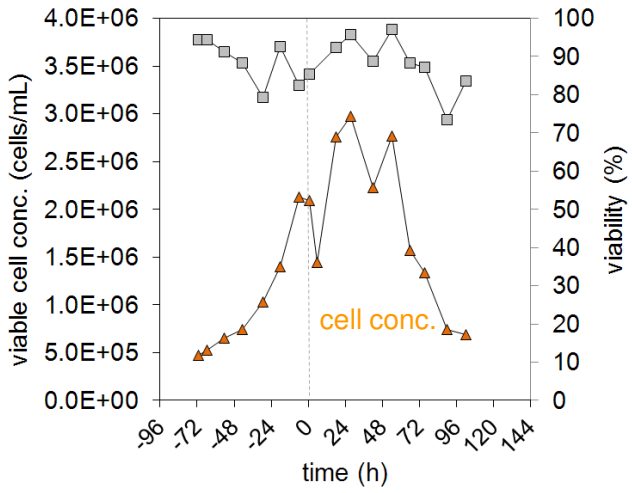
CAP in SFM: trypsin & medium exchange

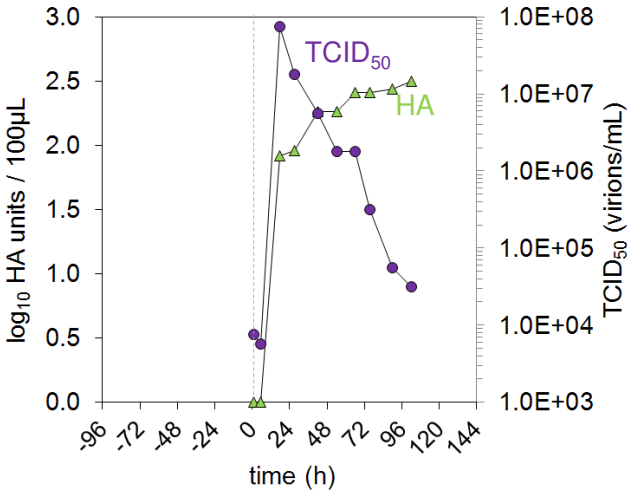


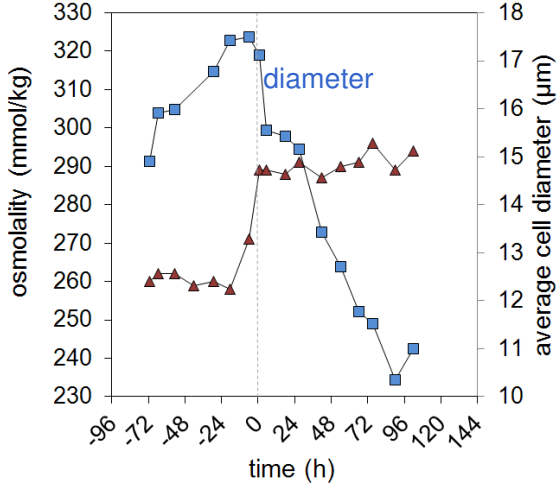
virus	experimental condition	cell concentration toi (x 10^6 cells/mL)	max HA (log_{10} HA units/100 µL)	cell spec. productivity (virions/cell)
A/PR/8-RKI$_{capa}$	without med. exch.	4.5	2.3	889
A/PR/8-RKI$_{capa}$	with med. exch.	5.0	3.2	6400
A/PR/8-RKI$_{capa}$	with 1:2 dilution	2.3	2.8	5478

CAP adapted A/PR/8/34 H1N1 influenza in CAP cells grown in PEM medium in shaker flasks

CAP in SFM in 1 L STR





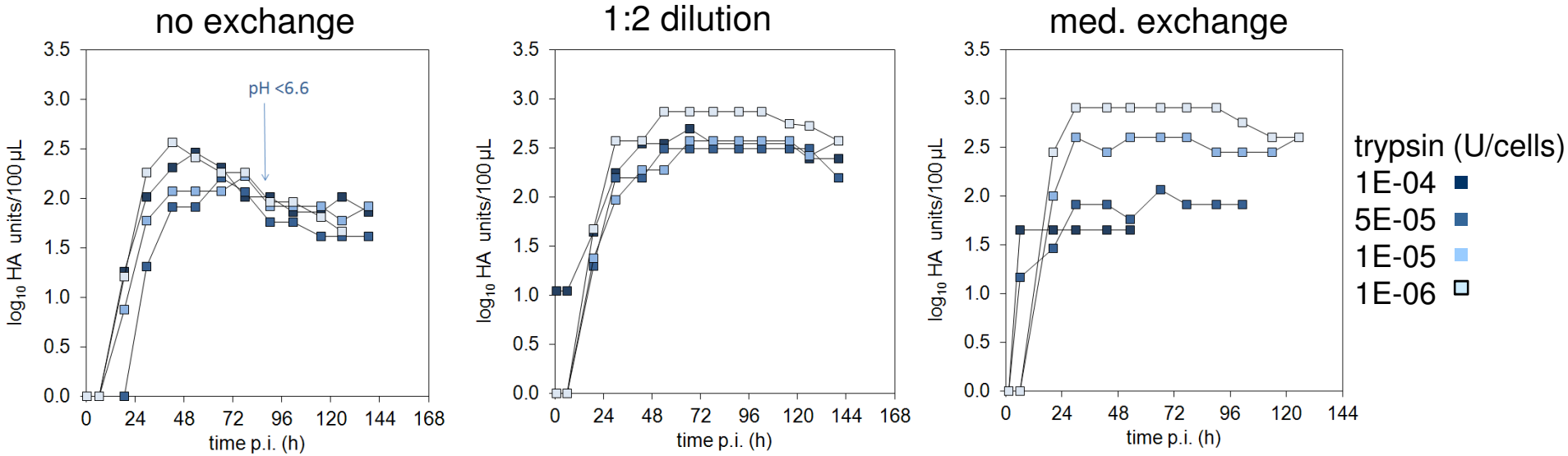


	CAP
medium	PEM
max cell concentration (x 10^6 cells/mL)	3.0
trypsin (x 10^{-5} U/cell)	3
moi	0.025
virus	A/PR/8-RKI$_{capa}$ (SEM293)
max HA (log_{10} HA units/100 µL)	2.5
cell spec. productivity (virions/cell)	2130

→ medium/conditions for virus production needs improvement (slime formation)

CAP adapted A/PR/8/34 H1N1 influenza in CAP cells grown in PEM medium in 1L biostat STR

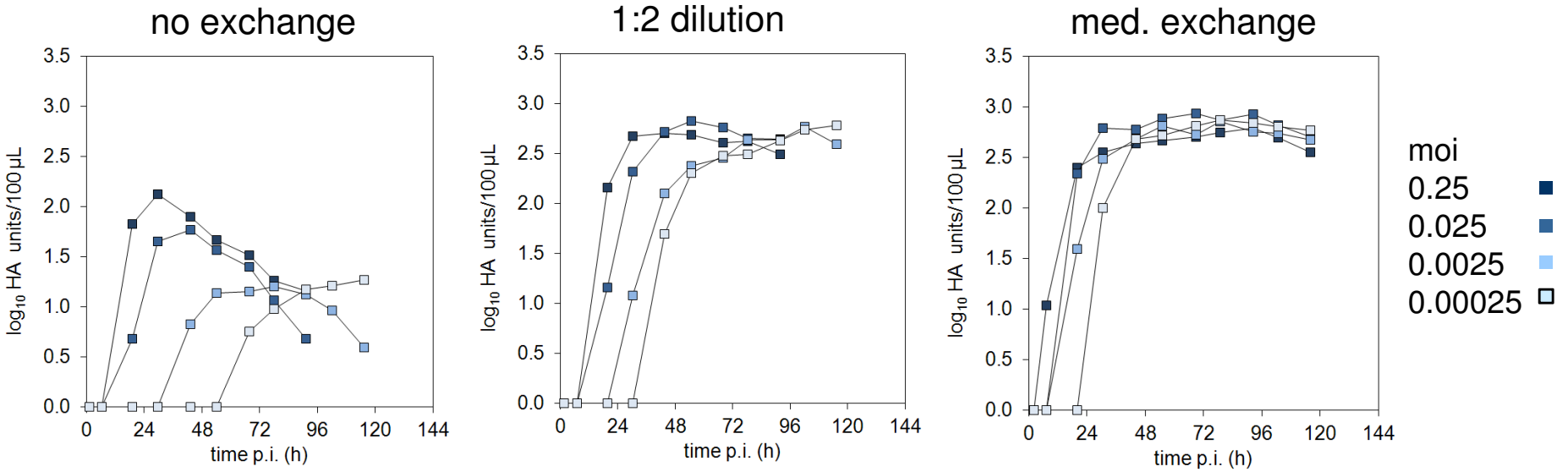
CAP in CDM: trypsin & medium exchange



virus	experimental condition	max cell concentration (x 10^6 cells/mL)	max HA (log_{10} HA units/100 µL)	cell spec. productivity (virions/cell)
A/PR/8-RKI$_{capa}$	without med. exch.	6.1	2.6	1210
A/PR/8-RKI$_{capa}$	with med. exch.	6.0	2.9	2720
A/PR/8-RKI$_{capa}$	with 1:2 dilution	6.6	2.9	2290

CAP adapted A/PR/8/34 H1N1 influenza in CAP cells grown in CDM2 medium (Teutocell) in shaker flasks, moi:0.025

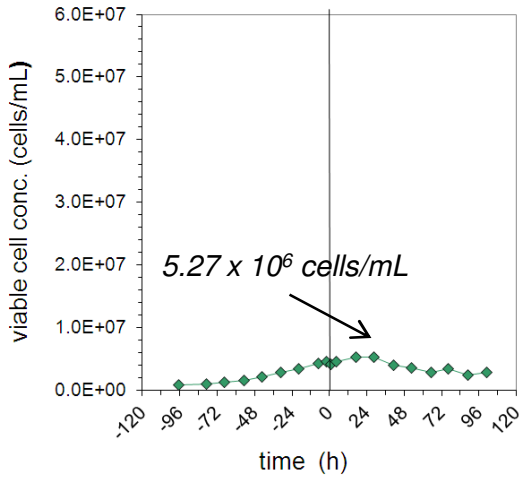
CAP in CDM: moi

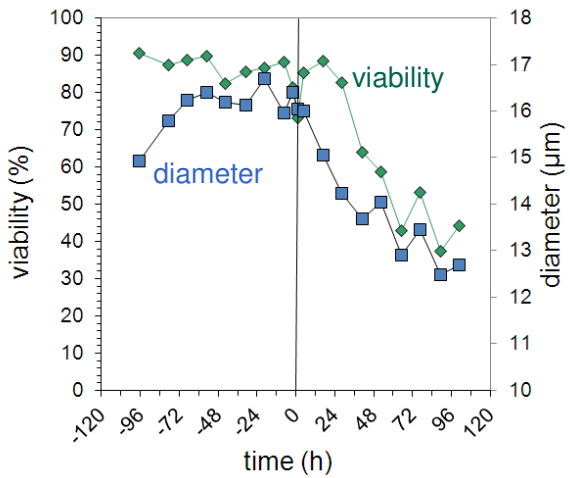


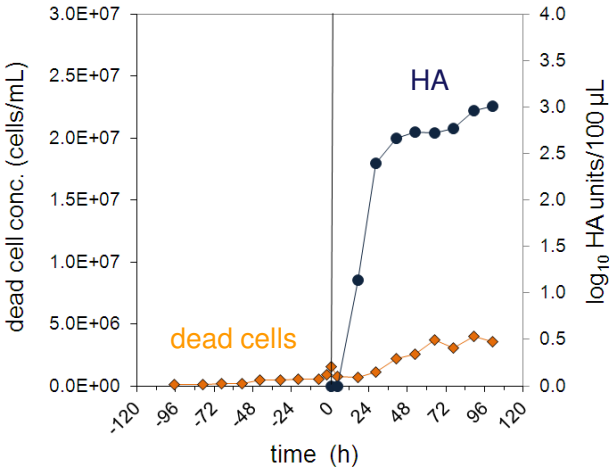
virus	experimental condition	max cell concentration (x 10^6 cells/mL)	max HA (log_{10} HA units/100 µL)	cell spec. productivity (virions/cell)
A/PR/8-RKI$_{capa}$	without med. exch.	5.7	2.1	463
A/PR/8-RKI$_{capa}$	with med. exch.	5.0	2.9	3140
A/PR/8-RKI$_{capa}$	with 1:2 dilution	6.4	2.8	2100

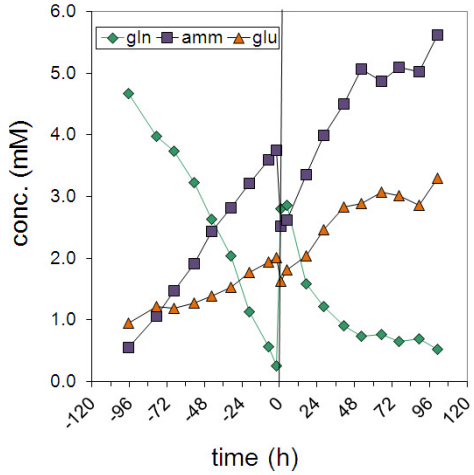
CAP adapted A/PR/8/34 H1N1 influenza in CAP cells grown in CDM2 medium in shaker flasks 1E-06 trypsin U/cells

CAP in CDM: 1L batch mode





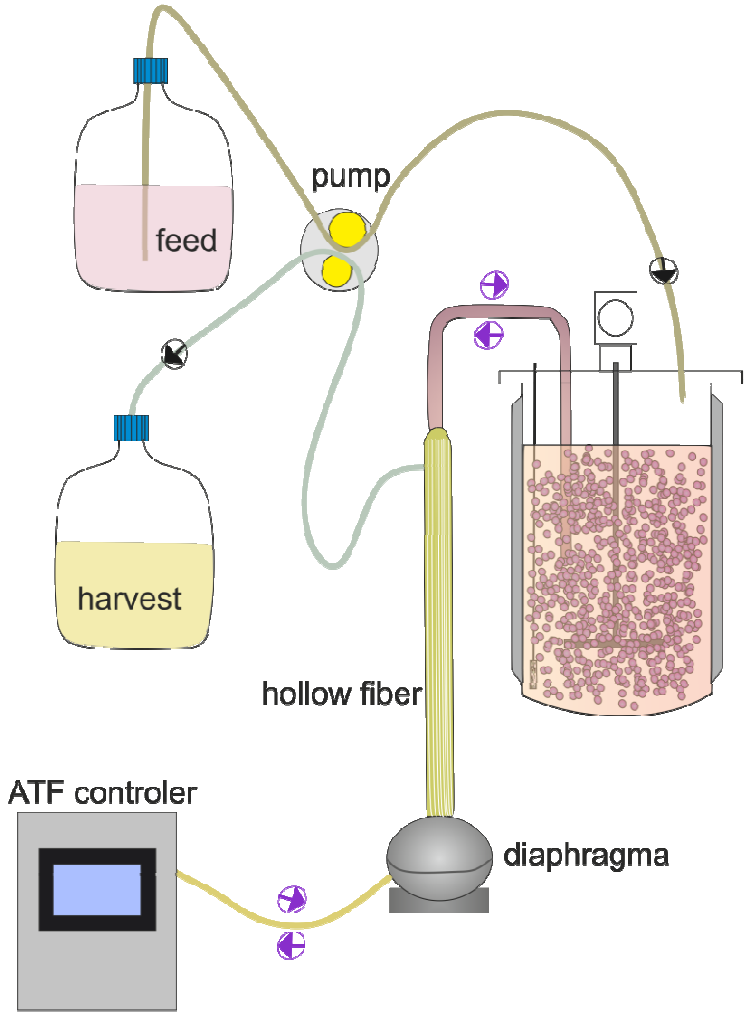


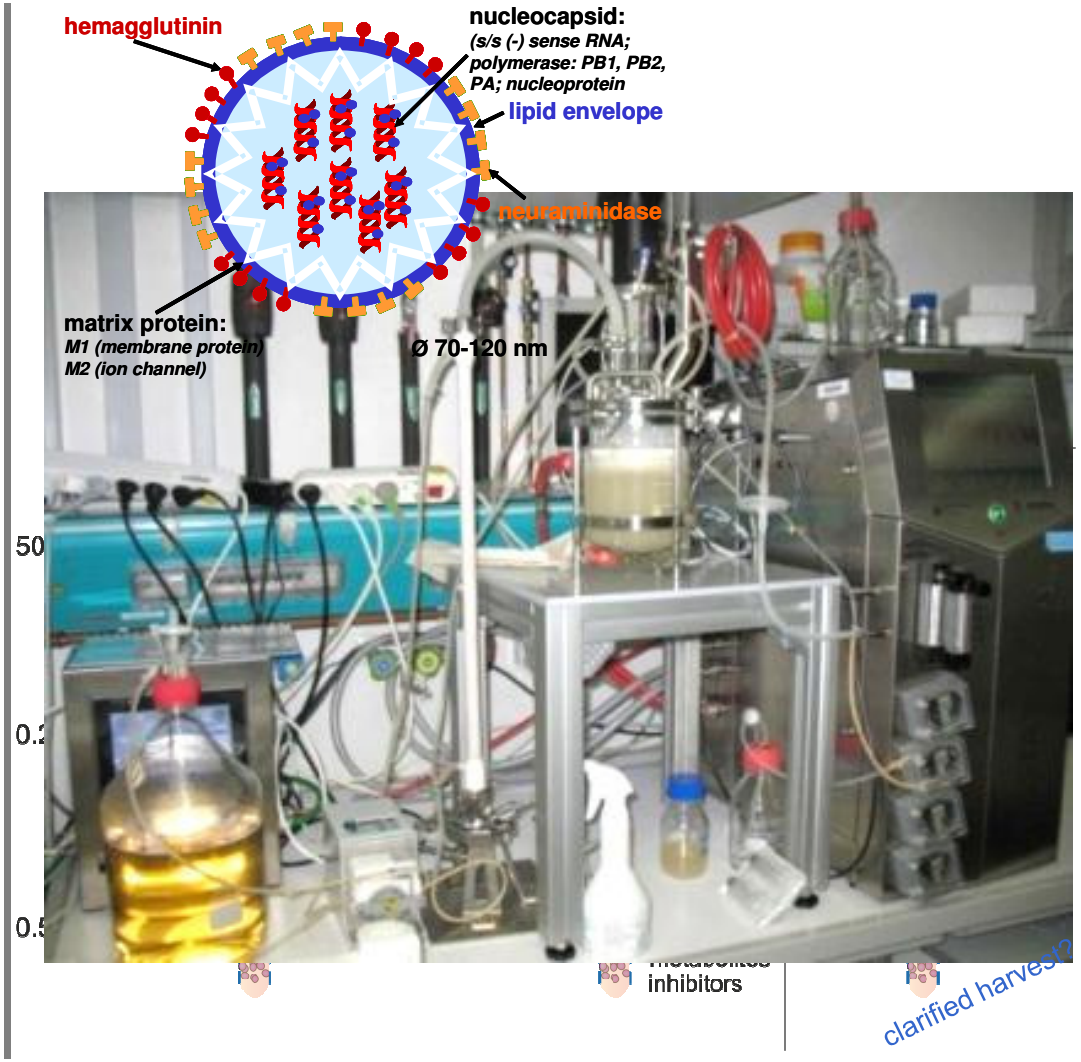


1:2 dilution at toi (B52)

virus	experimental condition	max cell concentration (x 10^6 cells/mL)	max HA (log_{10} HA units/100 µL)	cell spec. productivity (virions/cell)
A/PR/8-RKI$_{capa}$	with 1:2 dilution	5.3	3.0	3880
A/PR/8-RKI$_{capa}$	without med. exch.	4.0	2.0	537

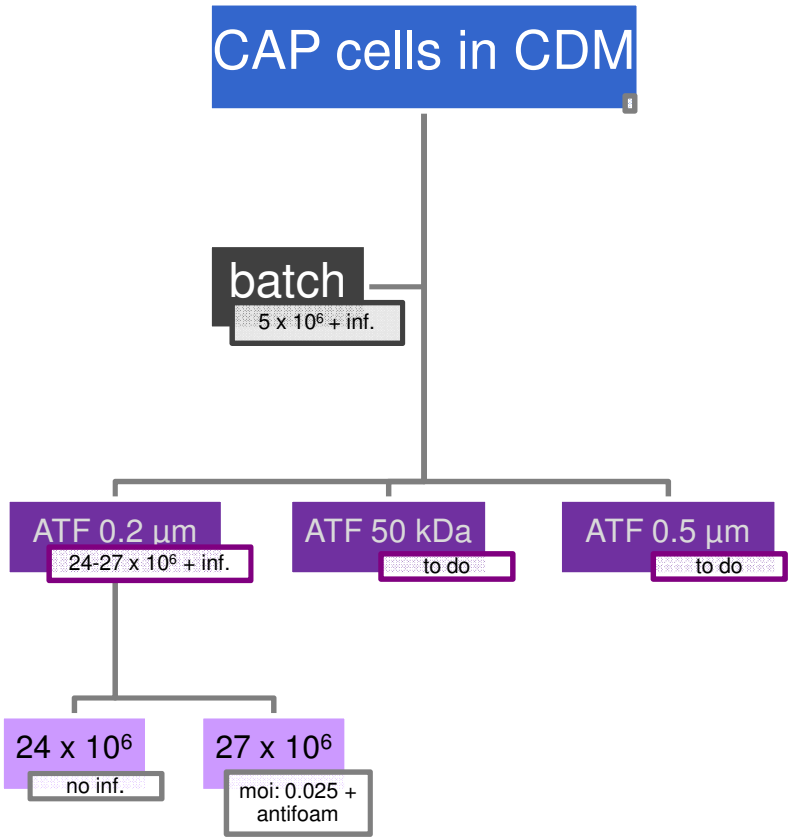
ATF for influenza virus production

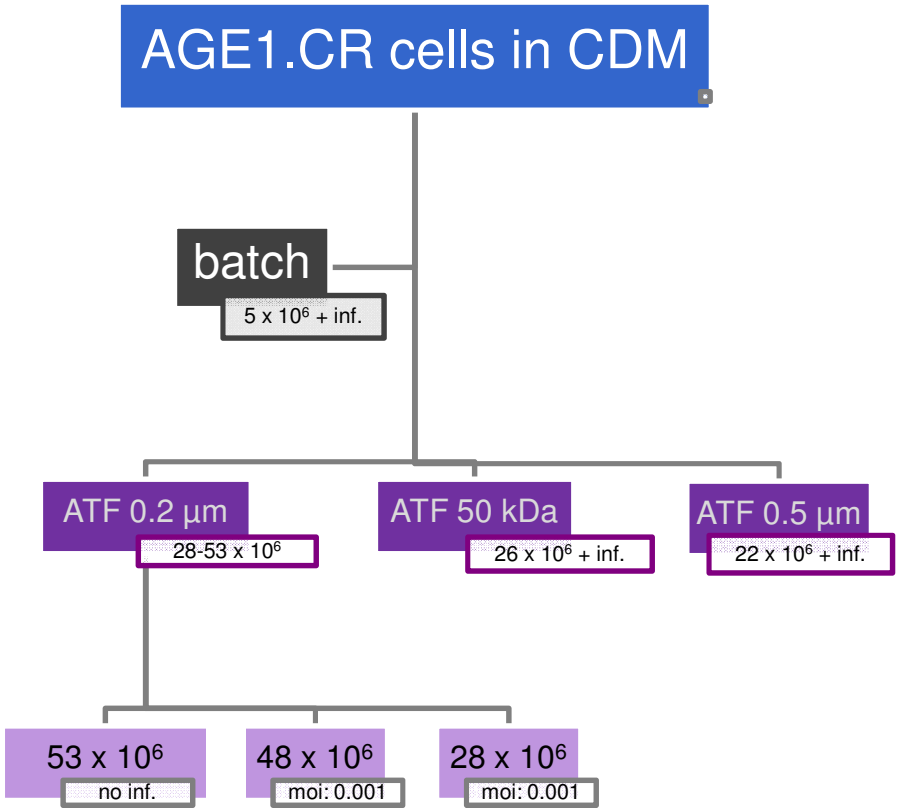




ALTERNATING TANGENTIAL FLOW TECHNOLOGY

ATF for influenza virus production



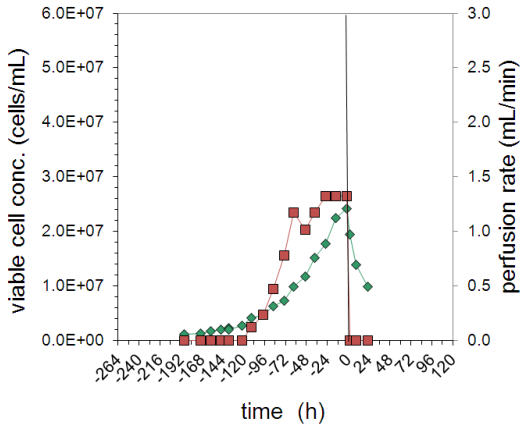


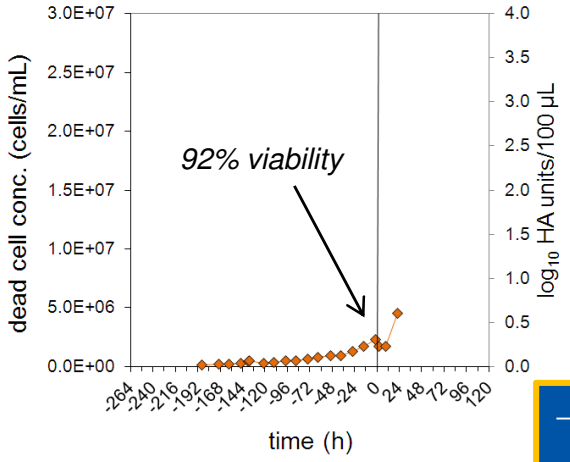
start: 1 x 10^6 cells/mL
perfusion rate manually adjusted according to metabolite conc.

1.75 h perfusion stopped for virus entry
adapted A/PR/8 (RKI) virus seed,
trypsin: 1 x 10^{-6} U/cells

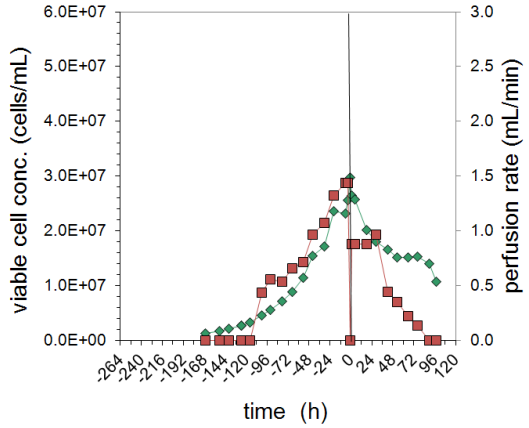
CAP at high cell densities (0.2 µm cut-off)...

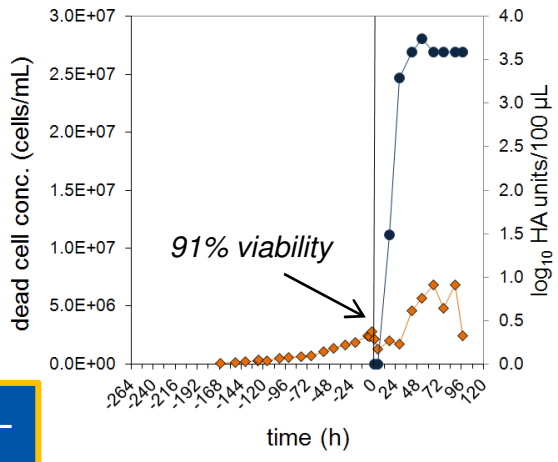
24 x 10^6 cells/mL, no virus (B53)





27 x 10^6 cells/mL (B58)





→ perfusion works up to 27 x 10^6 cells/mL
→ antifoam helps against slime formation
→ no virus in harvest

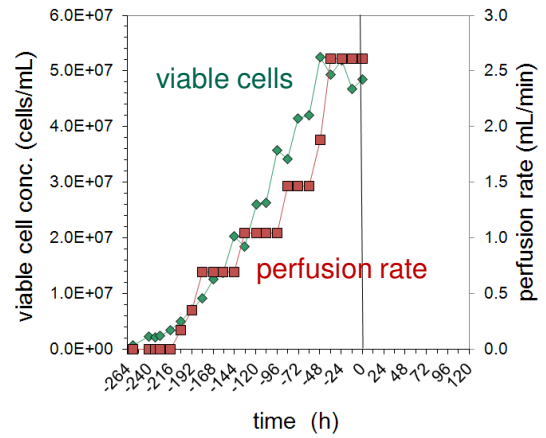
Yields for CAP

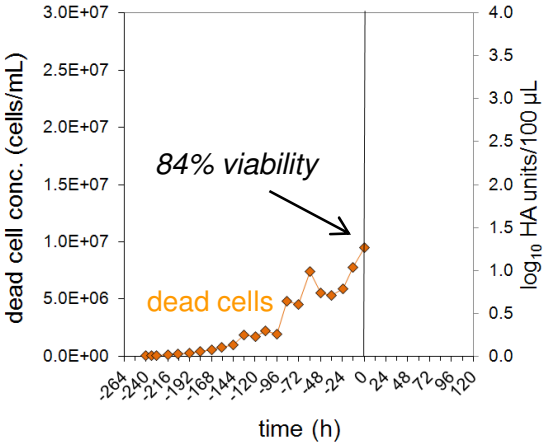
	batch (B52)	ATF (B53)	ATF (B58)
HF cut-off		0.2 µm	0.2 µm
passage nr	36	38	47
doubling time (d)	32.4	35.7	36.5
moi	0.025		0.025
seed virus	A/PR/8/34		A/PR/8/34
max. perfusion rate (mL/min)		1.3	1.4
total virus harvest (HA) (virions)	2.05E+13		9.89E+13
virus conc (HA) (virions/mL)	2.05E+10		1.10E+11
max HA (log HAU/100µL)	3.00		3.74
max TCID50 (virions/mL)	1.00E+09		
max. cell conc. (cells/mL)	5.27E+06	2.42E+07	2.69E+07
total viable cell numbers (cells)	5.27E+09	2.18E+10	2.42E+10
vol for cell growth (L)	1.0	6.7	5.9
time for cell growth (d)	4.0	10.7	7.0
time to harvest (d)	4.2		9.2
total vol. (L)	1.5	6.7	9.1
space-time-yield (virions/(d x L))	3.25E+12		1.18E+12
cell specific virus yield (virions/cell)	3883		4086
virus in harvest?			no
comment	1:2 dil., no pH control (V)	"slime"	antifoam

→ perfusion rate is not optimized: space-time-yields can be improved
→ cell specific virus yield is stable: no high cell density effect

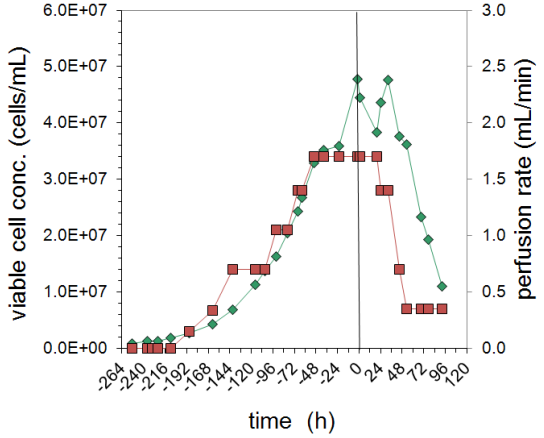
AGE1.CR at high cell densities: 0.2 µm cut-off

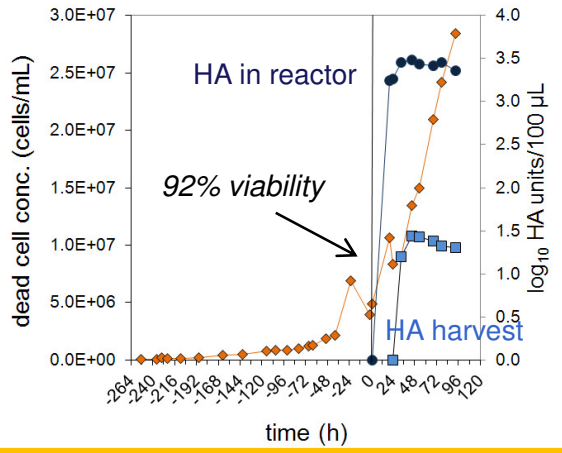
53 x 10^6 cells/mL, no virus (B47)



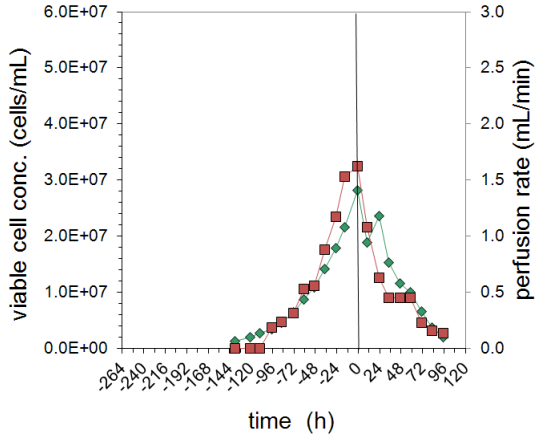


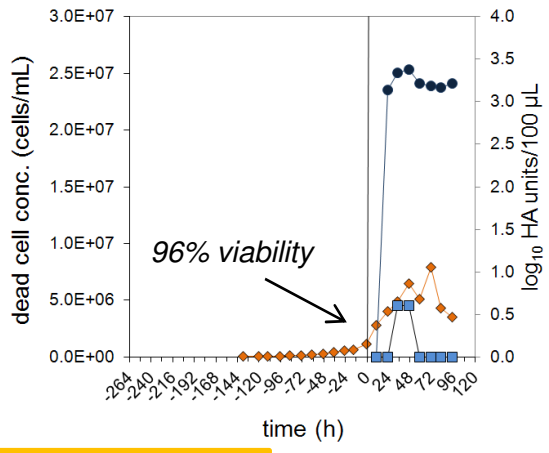
48 x 10^6 cells/mL (B44)





28 x 10^6 cells/mL (B51)



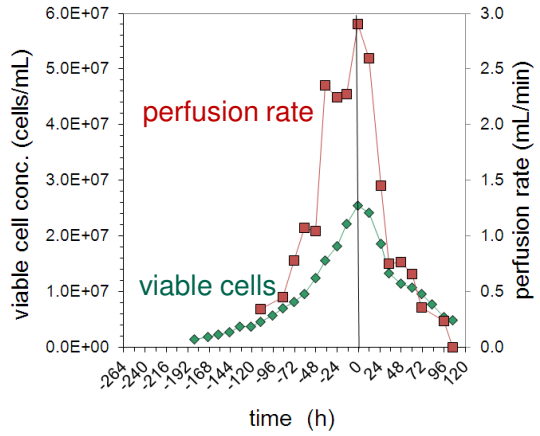


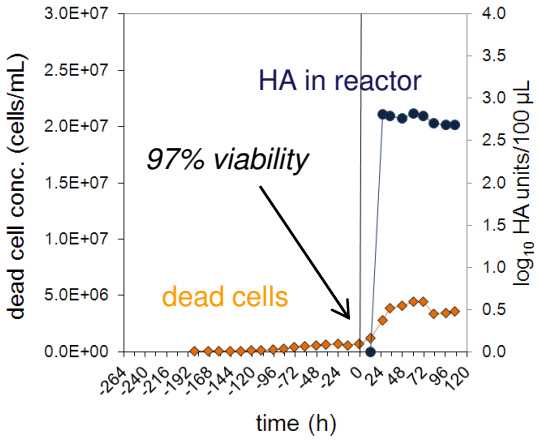
→ perfusion works up to 53 x 10^6 cells/mL with CD-U3 medium
→ virus in harvest

see also Poster 20

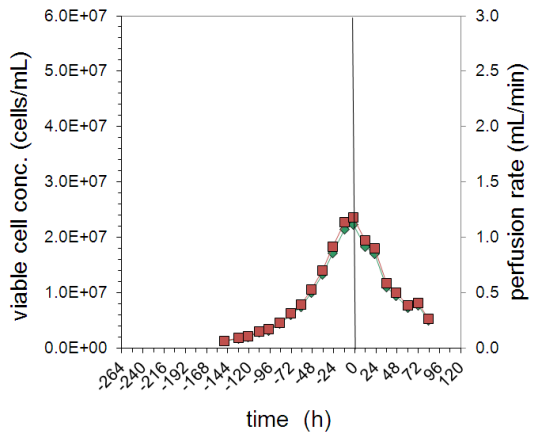
changing cut-off ...

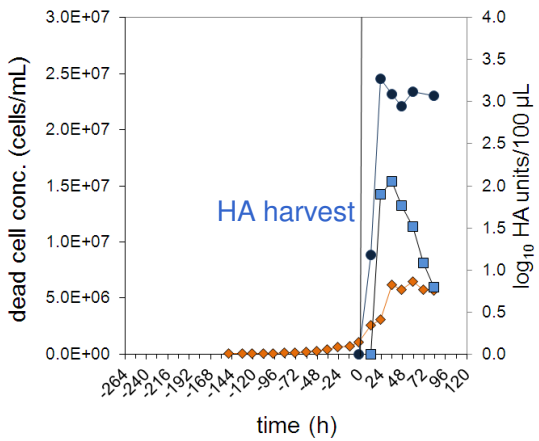
50 kDa
26 x 10^6 cells/mL (B54)



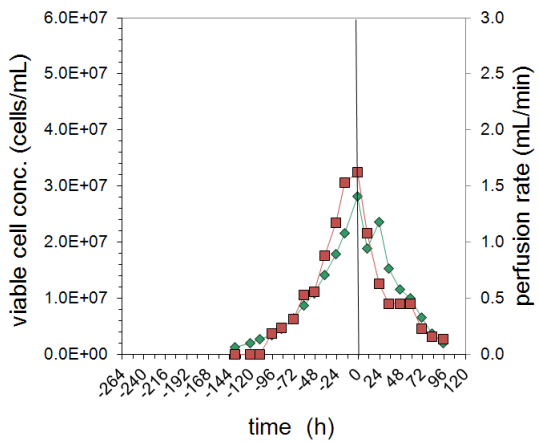


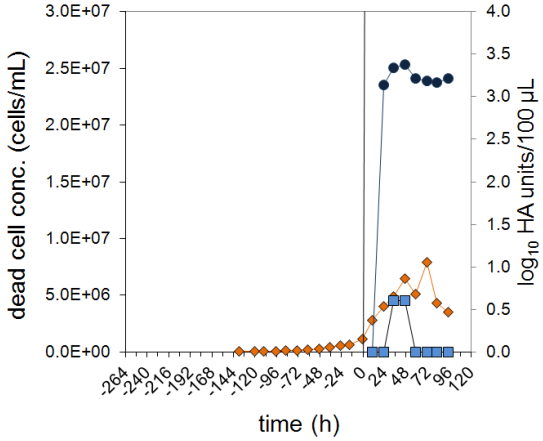
0.5 µm
22 x 10^6 cells/mL (B59)





0.2 µm
28 x 10^6 cells/mL (B51)





→ 50 kDa is holding back "virus titer influencing compounds"
→ 0.5 µm: important amount of virus in harvest

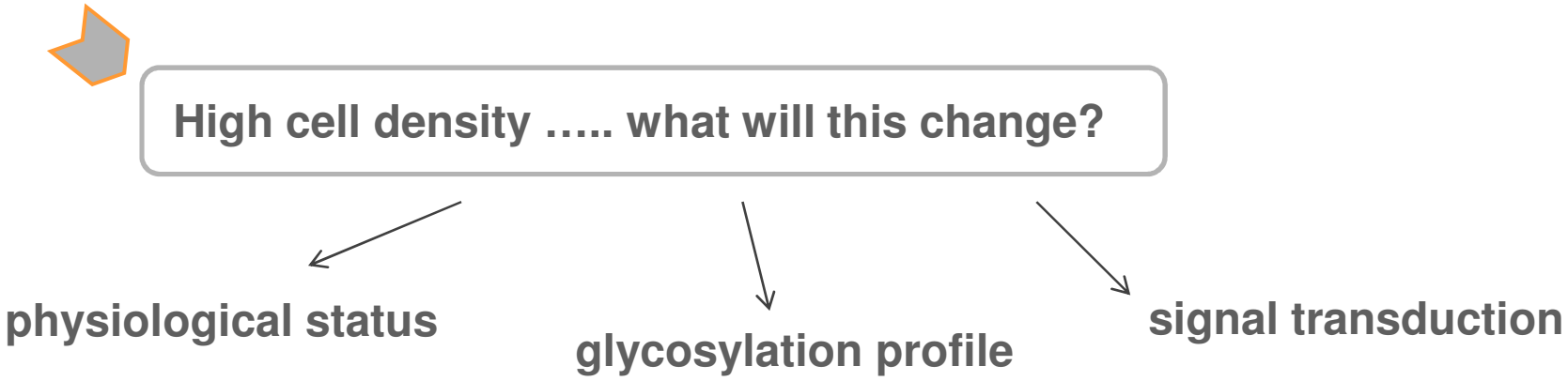
Yields for AGE1.CR

	batch (B50)	ATF (47)	ATF (B44)	ATF (B51)	ATF (B54)	ATF (B59)
HF cut-off		0.2 µm	0.2 µm	0.2 µm	50 kDa	0.5 µm
passage nr	106	35	77	106	52	
doubling time (d)	25	34	39	32	44	30
moi	0.025		0.001	0.001	0.001	0.001
seed virus	A/PR/8/34		A/PR/8/34	A/PR/8/34	A/PR/8/34	A/PR/8/34
max. perfusion rate (mL/min)		2.6	1.7	1.6	2.9	1.2
total virus harvest (HA) (virions)	7.43E+12		6.12E+13	3.67E+13	1.19E+13	3.46E+13
virus conc (HA) (virions/mL)	7.43E+09		2.19E+10	8.81E+09	1.32E+10	2.18E+10
max HA (log HAU/100µL)	2.57		3.48	3.38	2.82	3.27
max TCID50 (virions/mL)	3.20E+08		-	4.20E+09	1.00E+10	-
max. cell conc. (cells/mL)	5.75E+06	5.25E+07	4.77E+07	2.81E+07	2.55E+07	2.23E+07
total viable cell numbers (cells)	5.67E+09	4.73E+10	4.77E+10	2.25E+10	2.04E+10	2.01E+10
vol for cell growth (L)	1.0		13.0	5.8	6.4	4.7
time for cell growth (d)	1.90	10.70	10.50	5.60	7.70	6.2
time to harvest (d)	0.79		1.87	1.96	1.06	0.95
total time (d)	2.69		12.37	7.56	8.76	7.15
total vol. (L)	1.5		18.0	8.1	8.1	8.4
space-time-yield (virions/(d x L))	1.84E+12		2.75E+11	5.96E+11	1.68E+11	5.76E+11
cell specific virus yield (virions/cell)	1310		1283	1631	583	1724

→ perfusion rate is not optimized: space-time-yields can be improved
→ cell specific virus yield is stable: no high cell density effect

Summary

- ✓ CAP cells clearly show a potential for influenza vaccine production
- ✓ trypsin activity, virus medium and moi needs to be optimized
- ✓ HCD is possible with stable cell specific virus yield for AGE1.CR & CAP
- ✓ optimization of perfusion rate & medium can further improve space-time-yield
- ✓ additional options to select HF cut-off for process design



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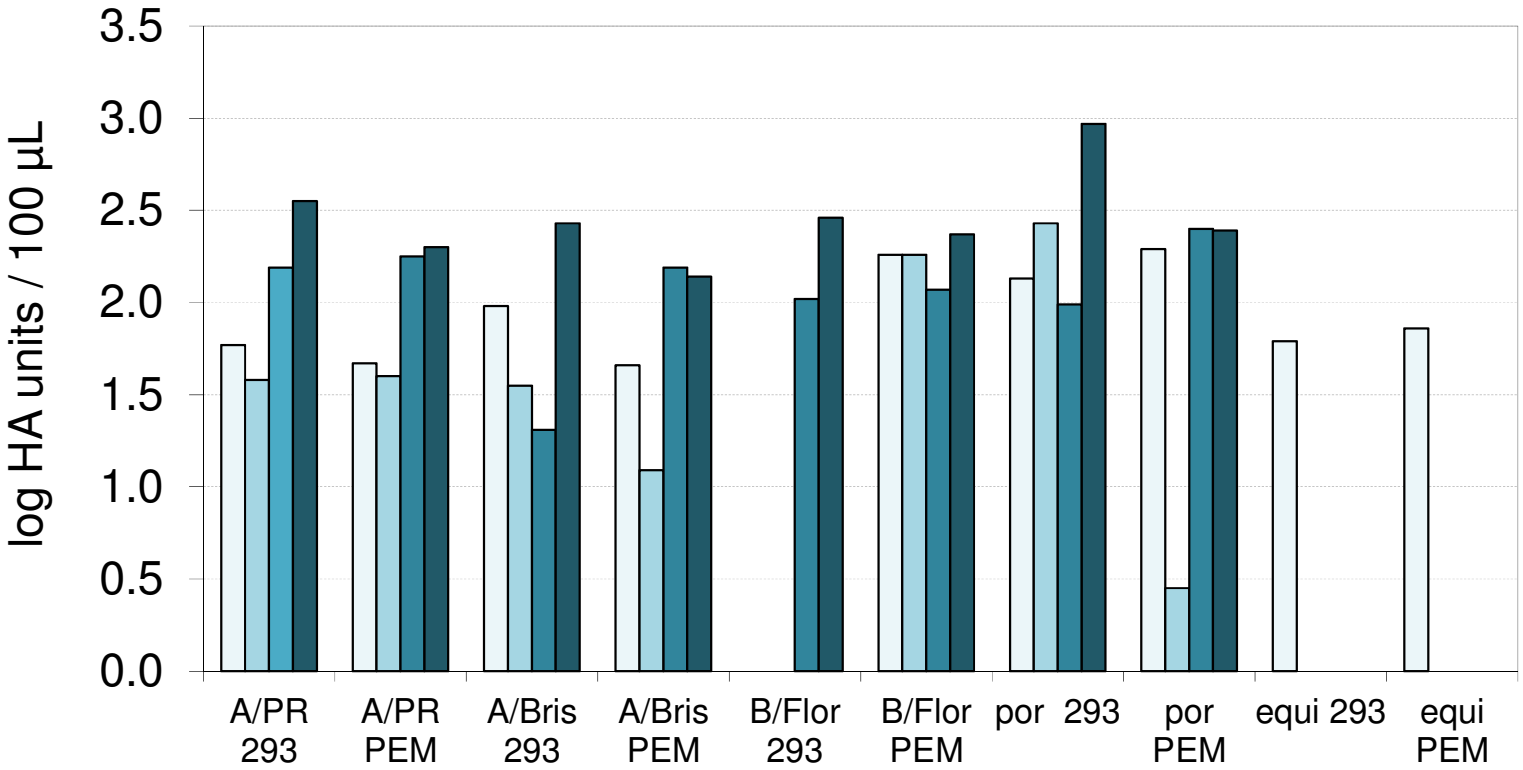
G. Schiedner (CEVEC)
S. Northoff (Teutocell)
C. Hetzel (IUL)

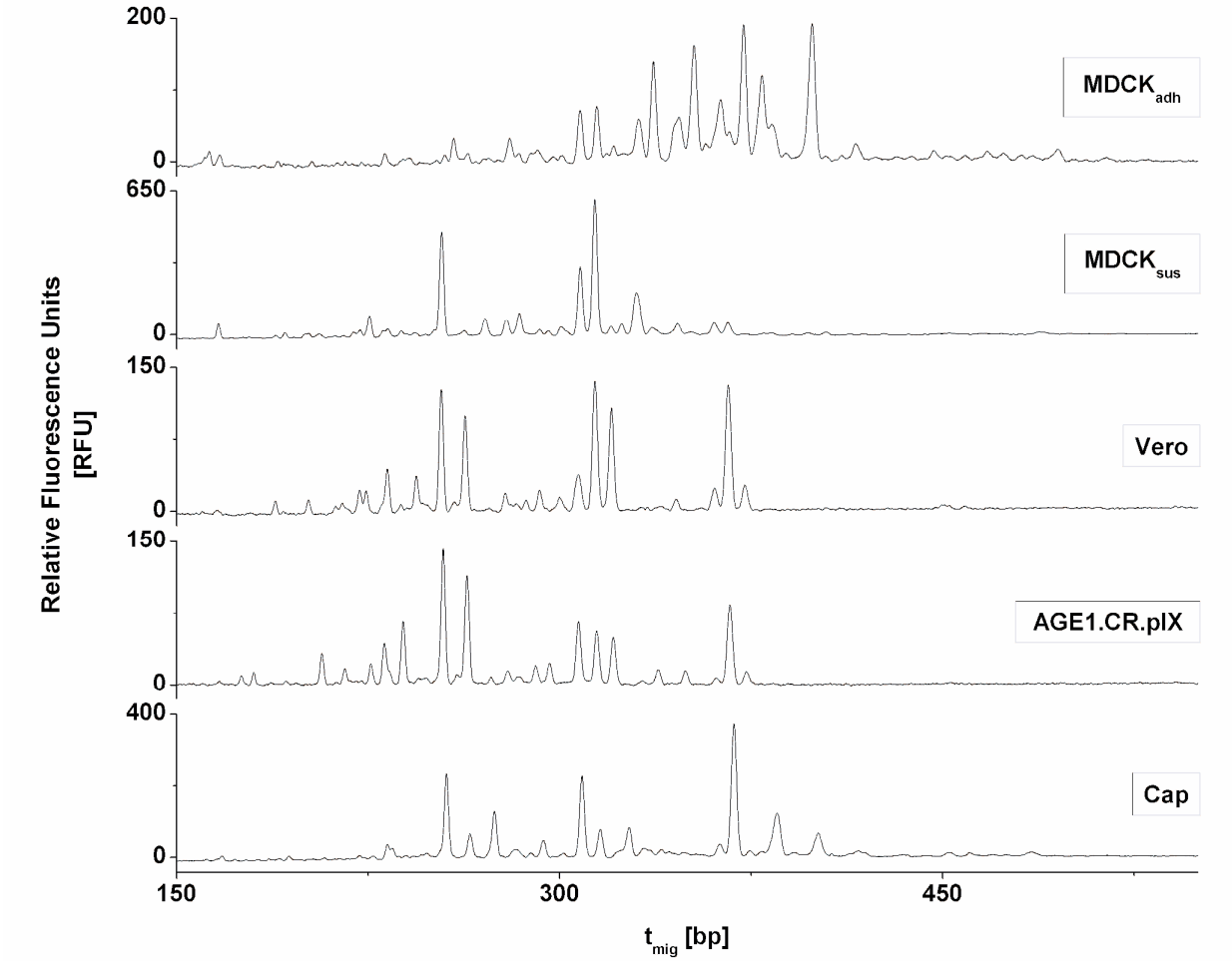
CAP: adaptation of different viruses



adaptation (4 passages) of MDCK cell-derived virus seeds to CAP cells
grown in PEM or SFM293 medium in shaker flasks with medium exchange
surface membrane expression of sialyloligosaccharides showed α2,3 and α2,6 sialic acids

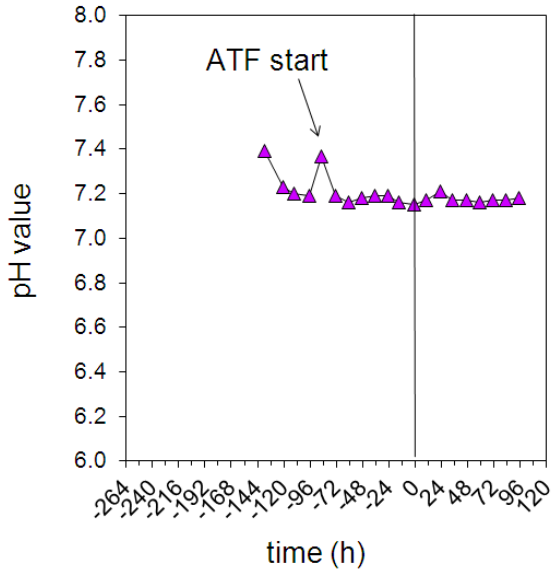
Genzel Y. et al. *submitted*

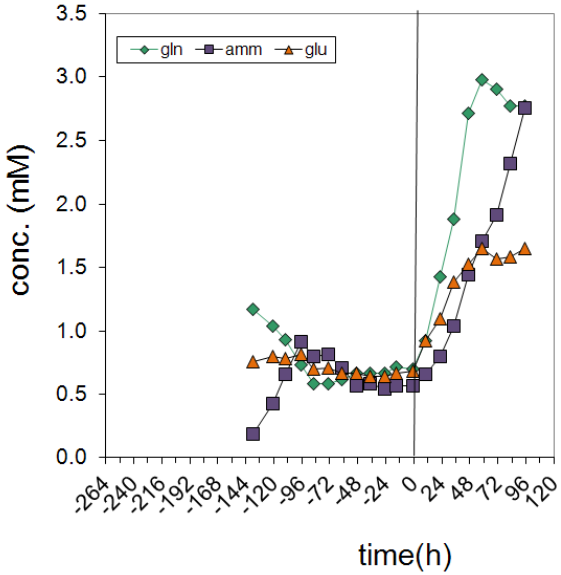
N-Glycan patterns for A/PR/8/34 HA

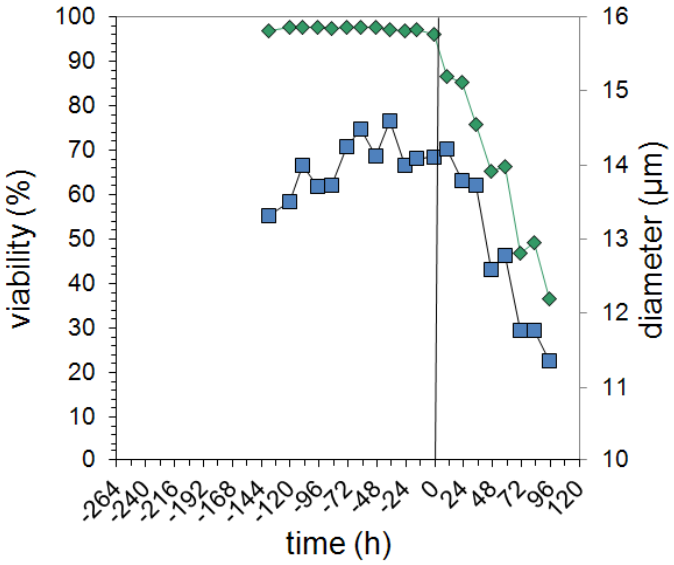


→ MDCK cells clearly have a different glycan pattern

other observations







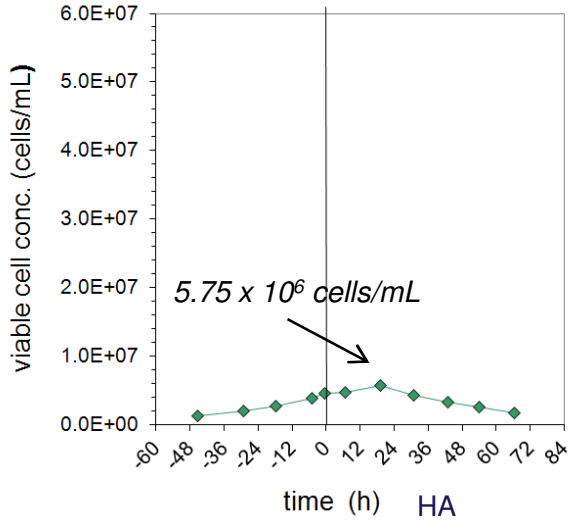
- pH increase with ATF start
- slow decrease at 50 kDa?

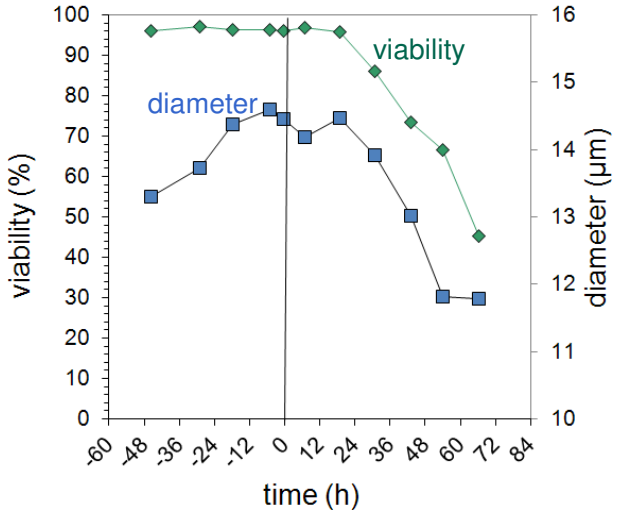
- metabolite plateau before toi
- strong increase of metabolites post infection?

- high viability
- smaller than CAP cells
- decrease of cell concentration during virus replication

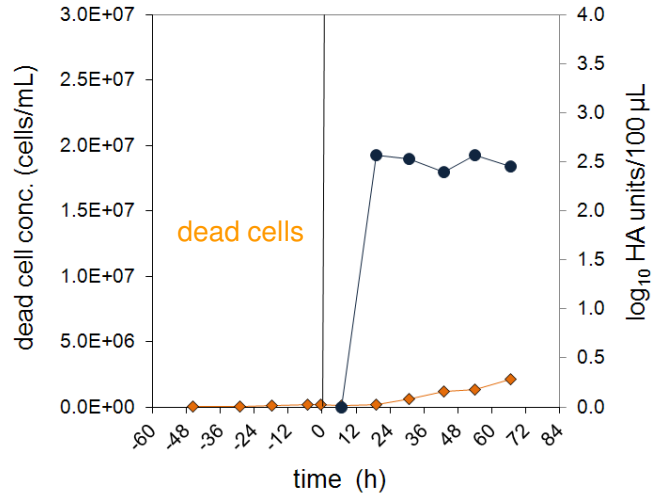
Cell growth of AGE1.CR in perfusion in CD-U3 medium (PAA) (B51)

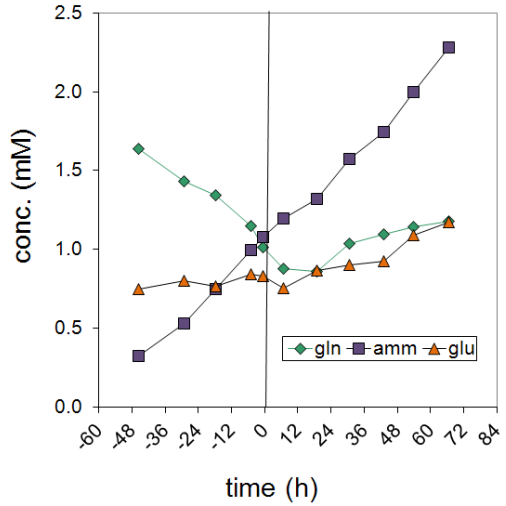
AGE1.CR in batch mode





cell spec. productivity (virions/cell)
1310





1:2 dilution at toi Cell growth of AGE1.CR in batch in CD-U3 medium (PAA) (B50)

Selected influenza strains

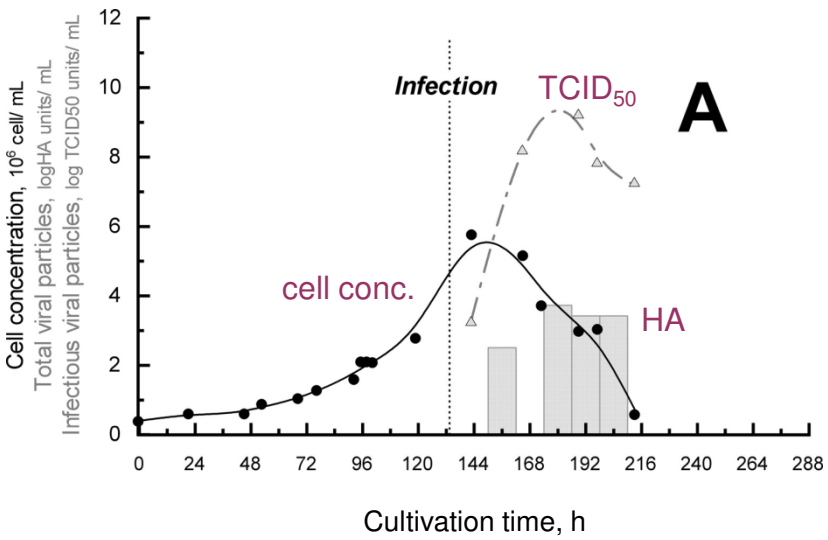
max. HA titer (log HA_{10} units/100µL)

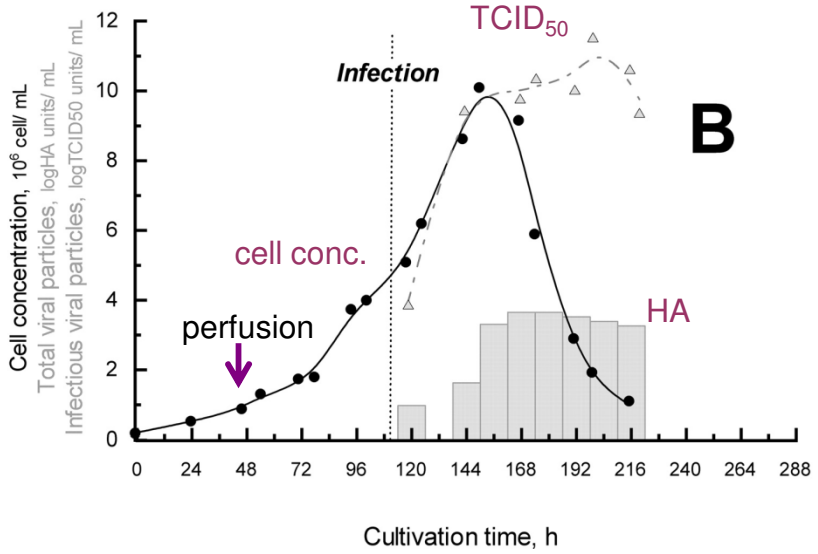
virus	$MDCK_{adh}$	Vero	MDCK.SUS2	AGE1.CR	AGE1.CR.pIX	HEK293	CAP
A/PR/8/34 H1N1 (RKI)	3.3	2.6	3.0	3.0	2.7	3.0	2.6
A/PR/8/34 H1N1 (NIBSC)	2.6	3.0	n.d.	2.1	2.1	n.d.	n.d.
A/Wisconsin/67/2005 H3N2-like HGR (NIBSC)	3.0	2.6	n.d.	2.5	2.4	n.d.	n.d.
A/Brisbane/59/2007 H1N1-like HGR (NIBSC)	3.0	n.d.	n.d.	2.6	2.3	n.d.	2.4
A/Uruguay/716/2007 H3N2-like HGR (NIBSC)	2.9	1.4	n.d.	-	-	n.d.	2.3
A/WSN/33 H1N1 (Freiburg)	3.3	-	n.d.	1.6	-	n.d.	n.d.
B/Malaysia/2506/2004 (NIBSC)	3.0	-	2.8	2.6	2.9	n.d.	n.d.
B/Florida/4/2006 (NIBSC)	2.8	-	n.d.	n.d.	n.d.	n.d.	2.5
delNS1 (A/PR/8/34) (Avir Greenhills Biotech.)	1.9	n.d.	n.d.	2.3	1.2	n.d.	n.d.
B/Lee/40 (ATCC)	n.d.	n.d.	n.d.	n.d.	n.d.	3.0	n.d.
Equine A/Newmarket/1/93 H3N8	2.8	n.d.	n.d.	2.0	1.6	n.d.	1.8
n.d. not determined; - no titer	*SCM*	*SCM adapted*	*CDM*	*SFM not adapted*	*SFM not adapted*	*SFM not adapted*	*SFM, adapted*

→ for each cell line potential is clearly shown, further optimization should follow ….

small scale cultivation: CR in AEM + gln in T-flasks, typical data for: MDCK & Vero in GMEM, MDCK.SUS2 in SMIF8, CAP in 293SFMII

HEK293 & perfusion



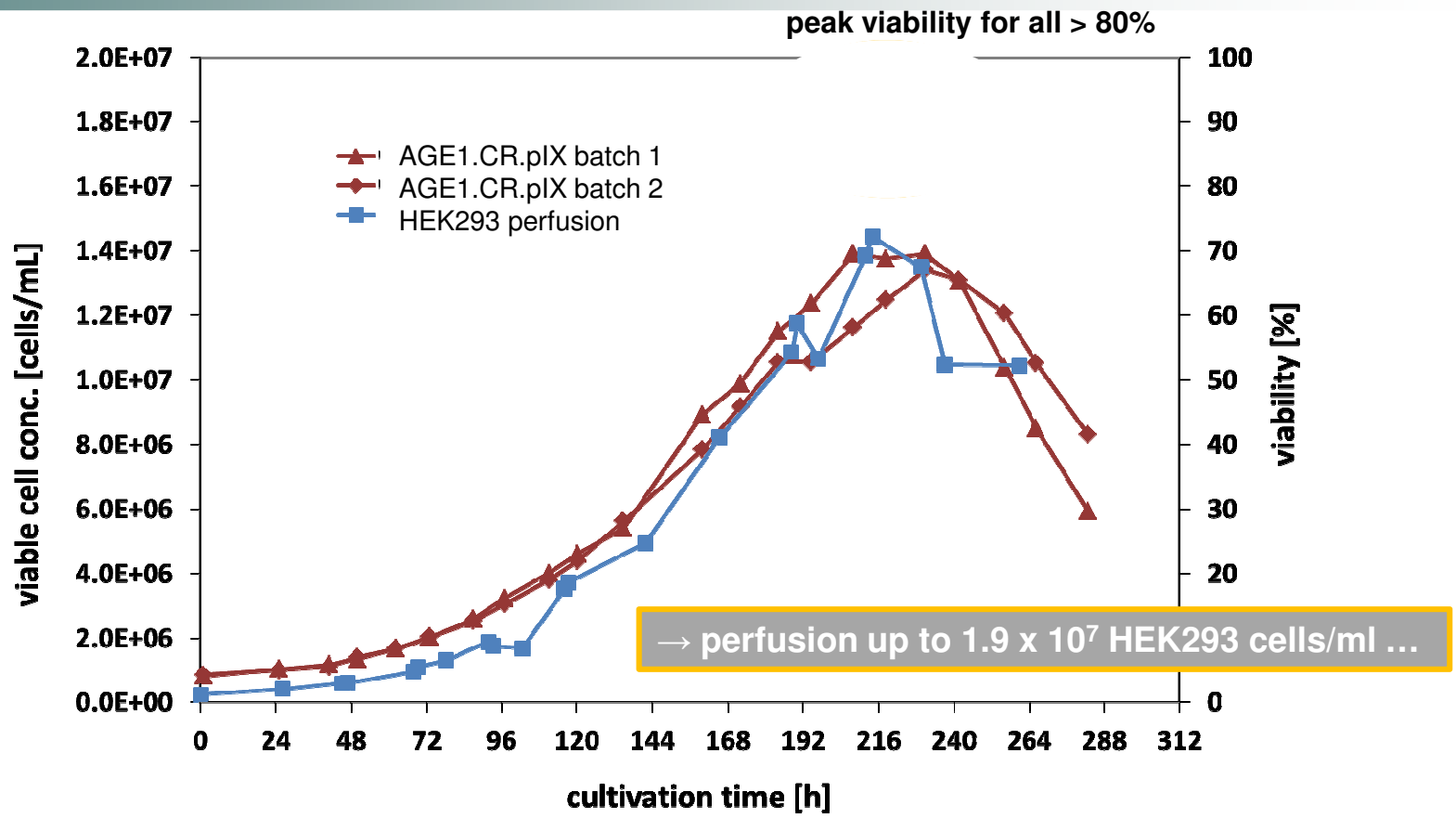


	perfusion	batch	batch
cell concentration at toi (x 10^6 cells/mL)	6.0	6.0	4.0
max. cell concentration (x 10^6 cells/mL)	10.1	5.8	4.4
max HA (log_{10} HA units/100 µL)	3.3	2.7	3.0
max. productivity of HA (log HA units/mL * mL harvest)	6.8	6.2	6.5
cell spec. productivity (virions/cell)	3951	1740	4683

HEK293-SF3F6 cells in 3L STR (Chemap, 85 rpm, HyClone SFM4 Transfx-293, acoustic filter), infl. A/PR/8/34 (moi: 0.001)

Le Ru A. et al. *Vaccine*. 2010; 28(21):3661-71. Petiot E. et al. *BMC Biotechnology* 2011, 11:84.

Potential of AGE1.CR.pIX and HEK293

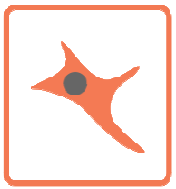


→ perfusion of AGE1.CR could lead to higher cell densities ??

Cell growth of AGE1.CR.pIX in batch against HEK293 cells grown in perfusion 3L STR

Le Ru A. et al. *Vaccine*. 2010; 28(21):3661-71. Petiot E. et al. *BMC Biotechnology*. 2011, 11:84.

History of CAP Cell Line Development

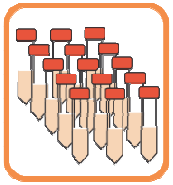


Primary Human Amniocytes

- Ethically obtained
- Non tumor origin
- Robust and scaleable
- Informed Consent

Immortalization

- Cloned adenoviral functions
- Non-oncogenic in humans



Master Cell Bank

- Fully certified
- Complete documentatio

Optimized Cell Clone

- Transient protein expression
- Stable protein expression
- Stable and fast growth
- Suspension