# The relevance of damage clustering on the $\mu$m and nm scale for the quantitative prediction of radiation effects

**M. Scholz**

T. Friedrich, F. Tommasino, L. Herr, U. Scholz, A. Hufnagl, M. Durante

GSI Darmstadt

- Role of Giant-Loop chromatin organization
- Application in Local Effect Model
- Rejoining kinetics after high-LET radiation
- Application to photon radiation: GLOBLE
- Cell cycle variation of radiosensitivity
- Application to ultrasoft X-rays
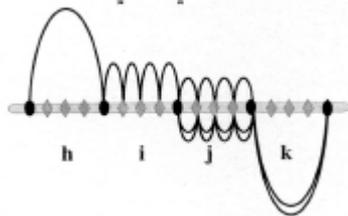
# Basic Idea of Local Effect Model

Biological response of cells is determined by the pattern of induced DSB, i.e. the number and their complexity (clustering)

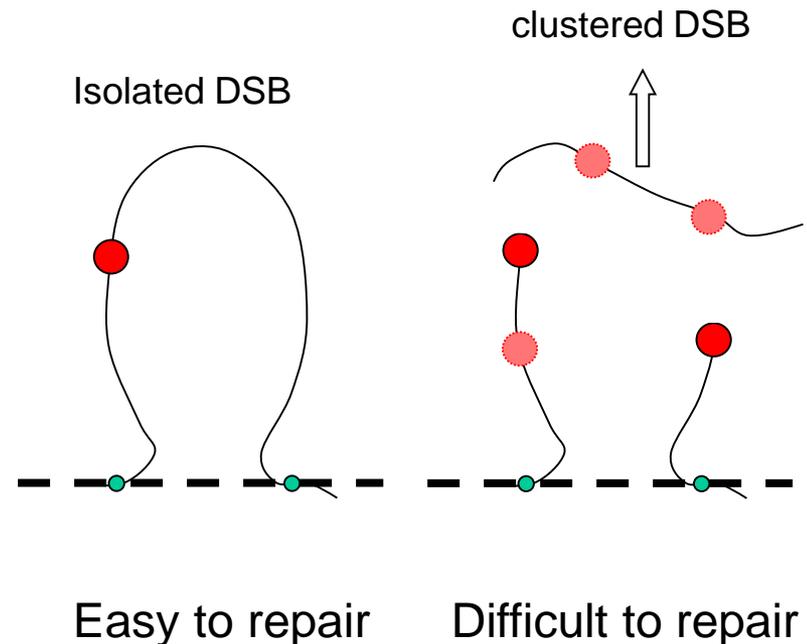Complexity of DSB can be defined in relation to chromatin structure

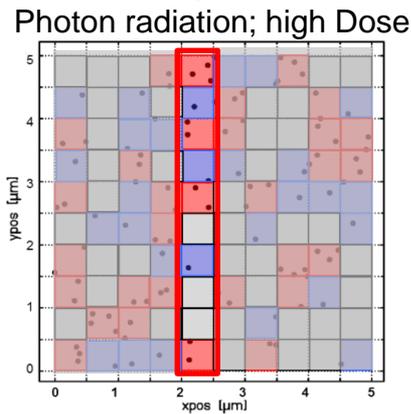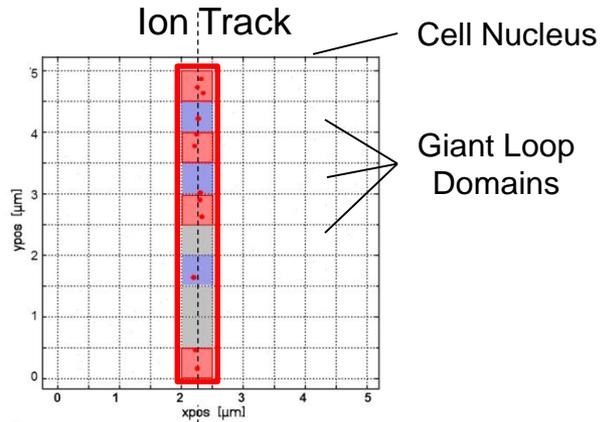Higher order chromatin organization: Giant Loops (~ 2Mbp, ~0.5$\mu$m)
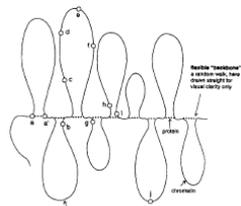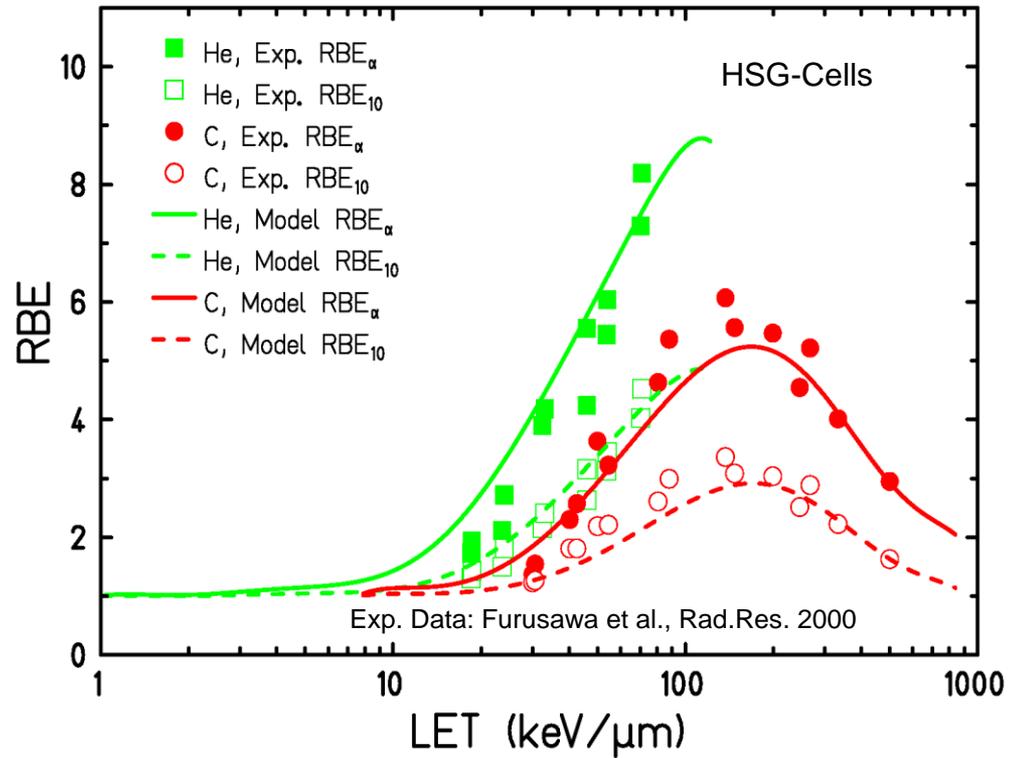
Yokota et al. JCB 1995

Johnston et al., Rad.Res. 1998

clustered DSB

Isolated DSB

Easy to repair          Difficult to repair

# Concept of LEM

Ion Track

Cell Nucleus

Giant Loop Domains

Photon radiation; high Dose

HSG-Cells

Exp. Data: Furusawa et al., Rad.Res. 2000

- He, Exp. RBE$_\alpha$
- He, Exp. RBE$_{10}$
- C, Exp. RBE$_\alpha$
- C, Exp. RBE$_{10}$
- He, Model RBE$_\alpha$
- He, Model RBE$_{10}$
- C, Model RBE$_\alpha$
- C, Model RBE$_{10}$

RBE

LET (keV/µm)

$n_{DSB}=0$

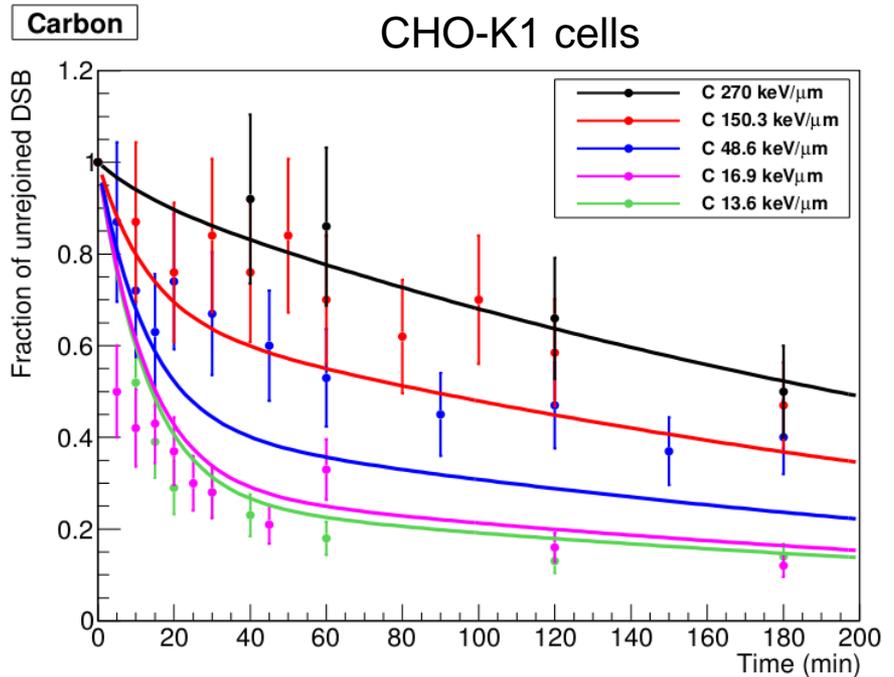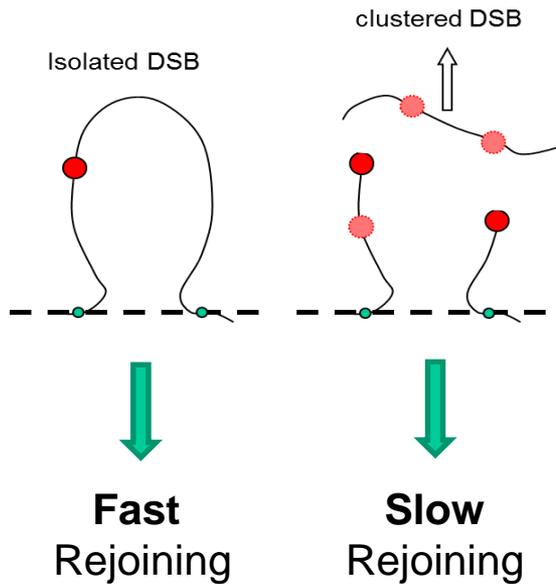$n_{DSB}=1$: isolated DSB

$n_{DSB}\geq2$: clustered DSB

Elsässer et al., Int. J. Radiat. Oncol. Biol. Phys. 2010

# Further support for the relevance of iDSB and cDSB?

# Rejoining of DSB: high-LET radiation



Isolated DSB

clustered DSB

**Fast** Rejoining

**Slow** Rejoining

Carbon — CHO-K1 cells

C 270 keV/μm
C 150.3 keV/μm
C 48.6 keV/μm
C 16.9 keV/μm
C 13.6 keV/μm

$$U(t) = \frac{n_i}{n_i + n_c} \exp(-\frac{\ln(2)}{\tau_{fast}} t) + \frac{n_c}{n_i + n_c} \exp(-\frac{\ln(2)}{\tau_{slow}} t)$$

$n_i$: # DSB in isolated DSB
$n_c$: # DSB in clustered DSB

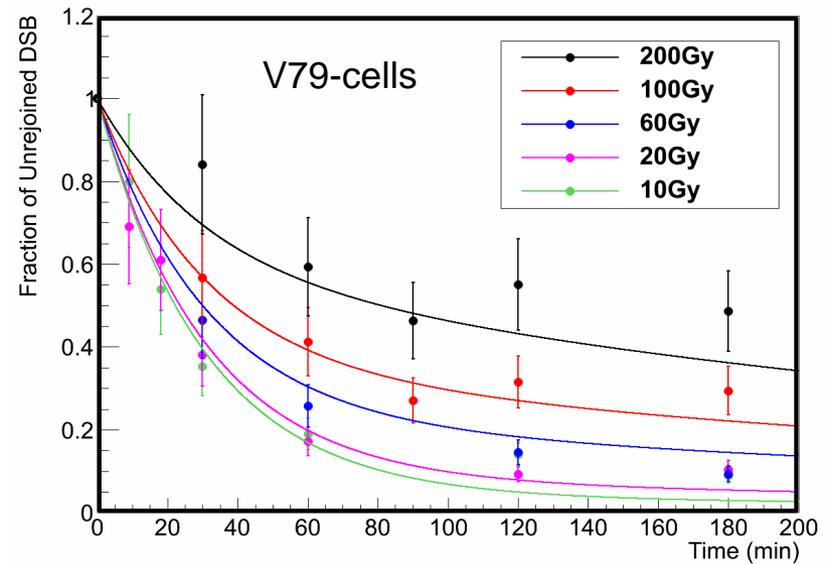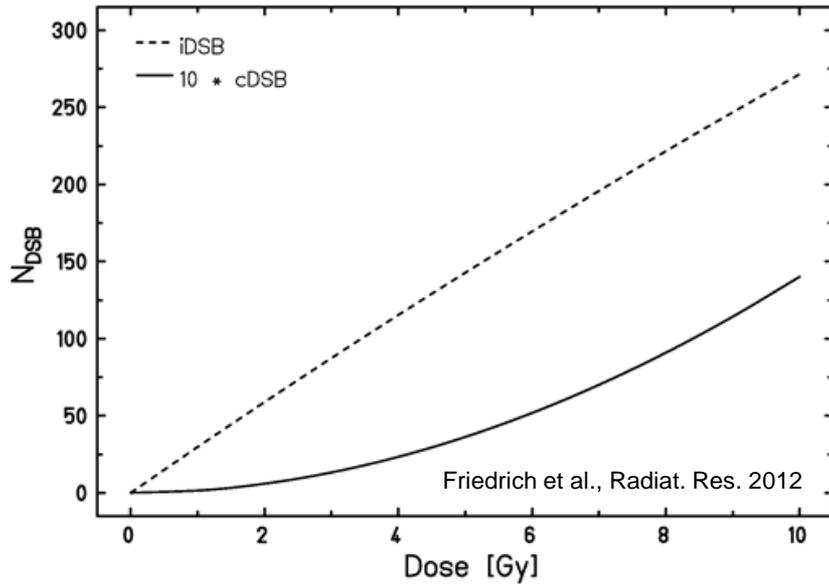Local Effect Model ➡ $n_i$, $n_c$

Simultanous Fit ➡ $\tau_{fast}$: 9±1 min   $\tau_{slow}$: 220±16 min

Tommasino et al., Radiat. Res. 2013

Photon radiation:

Increasing fraction of cDSB with increasing dose

➡ Increased fraction of slow rejoining?!



Friedrich et al., Radiat. Res. 2012



V79-cells
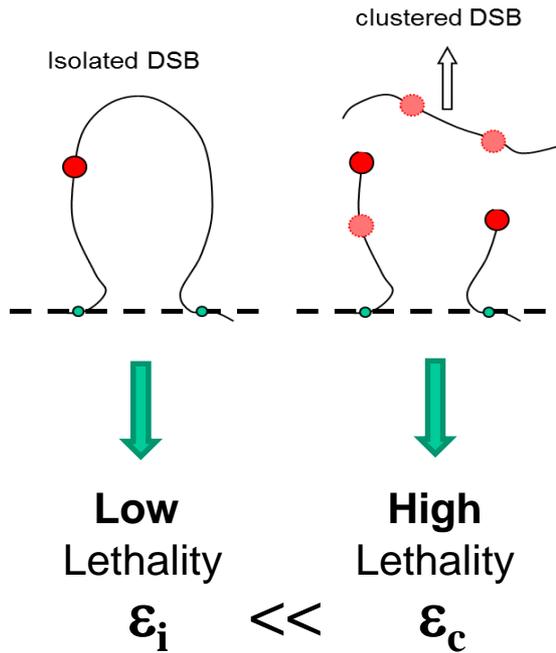
Simultanous Fit

➡ $\tau_{fast}$: 21±2 min  $\tau_{slow}$: 256±46 min

Poisson statistics ➡ $n_i$, $n_c$

**Symbols :** Exp. data
(Cucinotta et al,
Int J Rad Biol, 2000)

**Lines**: Model predictions
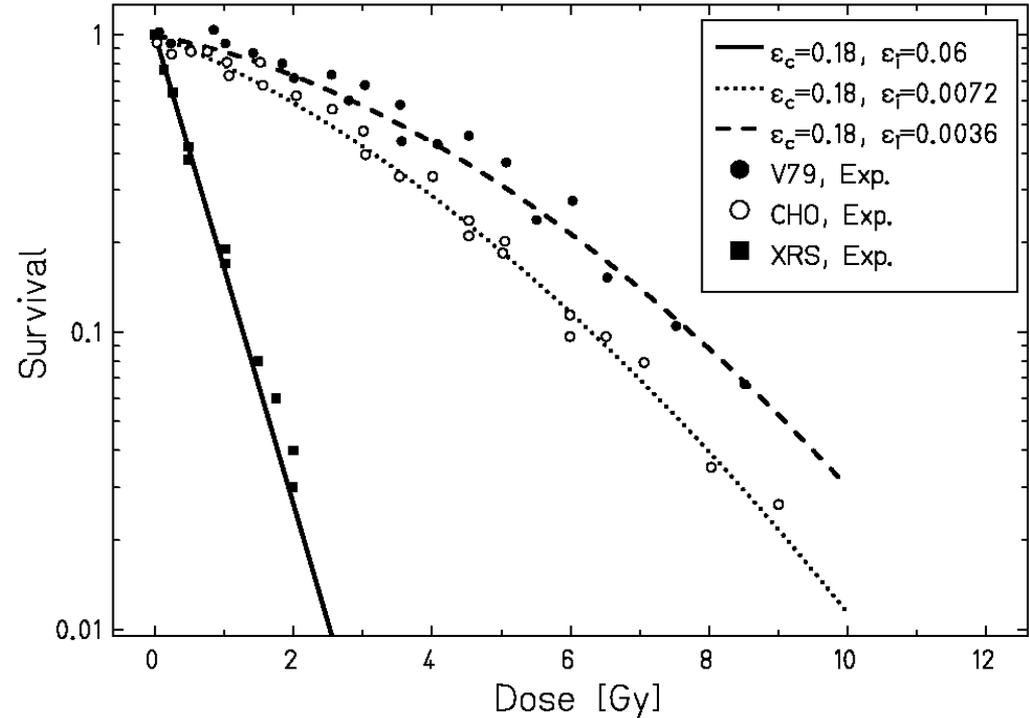(Tommasino et al., Radiat.
Prot. Dosim. 2015)

# Giant LOoop Binary LEsion (GLOBLE) model: application to cell survival after photon radiation



Isolated DSB

clustered DSB

**Low** Lethality

**High** Lethality

$\varepsilon_i \quad << \quad \varepsilon_c$

Poisson statistics $\rightarrow$ $n_i$, $n_c$

$$S = e^{-N_{lethal}} = e^{-(n_i \varepsilon_i + n_c \varepsilon_c)}$$
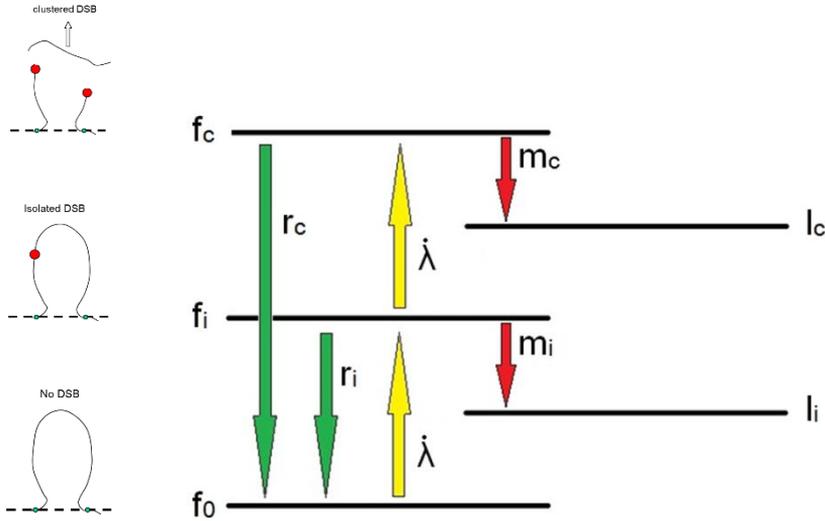
$$= e^{-(\alpha D + \beta D^2)}$$



Conversion:
$$\varepsilon_i = \frac{\alpha}{\alpha_{DSB}} \qquad \varepsilon_c = 2\frac{N_L \beta + \alpha_{DSB}\alpha}{\alpha_{DSB}^2}$$

Friedrich et al., Radiat. Res. 2012

clustered DSB

Isolated DSB

No DSB

$f_c$  $r_c$  $\dot{\lambda}$  $m_c$  $l_c$

$f_i$  $r_i$  $\dot{\lambda}$  $m_i$  $l_i$

$f_0$

**Lower effectiveness** of „cDSB", if DSB are separated in time!

$$\varepsilon_c \rightarrow 2 \cdot \varepsilon_i$$

$$\frac{df_0}{dt} = -\dot{\lambda}f_0 + r_i f_i + r_c f_c \qquad \frac{dl_i}{dt} = m_i f_i$$

$$\frac{df_i}{dt} = \dot{\lambda}f_0 - (\dot{\lambda} + r_i + m_i)f_i \qquad \frac{dl_c}{dt} = m_c f_c.$$

$$\frac{df_c}{dt} = \dot{\lambda}f_i - (r_c + m_c)f_c$$



MT-cells
$\tau_{fast}$: 5.2min

90 Gy/h
24 Gy/h
8.4 Gy/h
4.56 Gy/h
0.96 Gy/h



MT-cells
$\tau_{fast}$: 16.8 min

5 Gy
6 Gy

Time of separation [h]
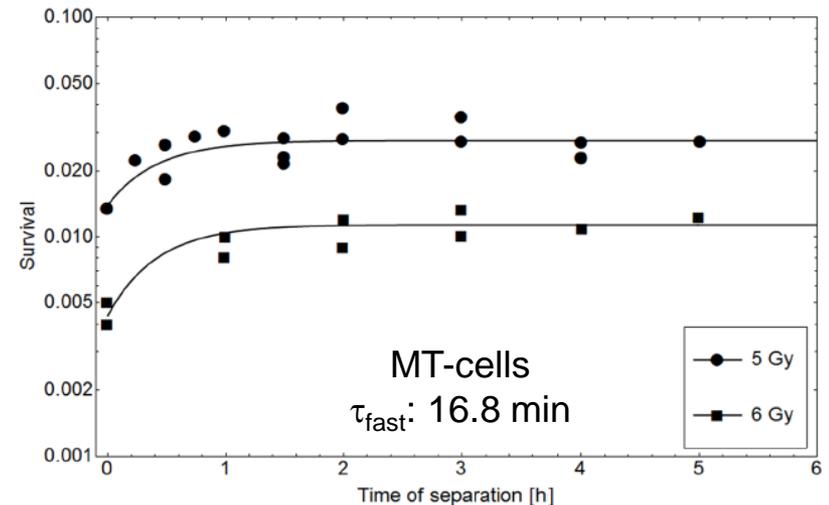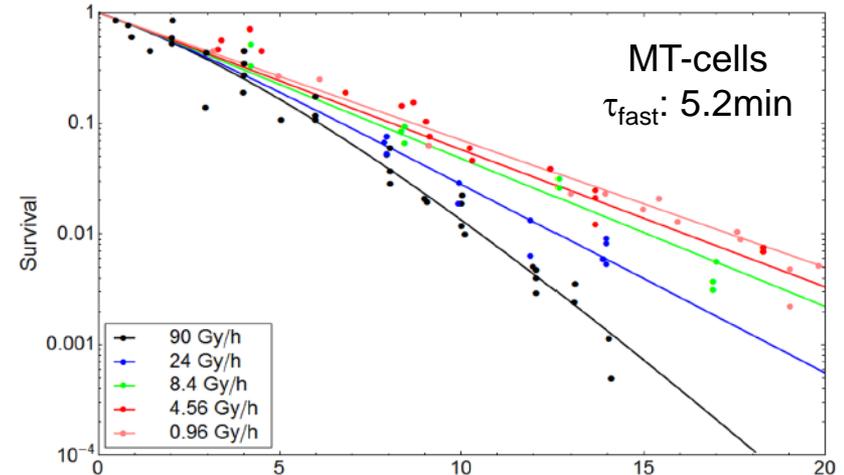
Herr et al., PLOS One 2014

# Cell cycle dependent radiosensitivity
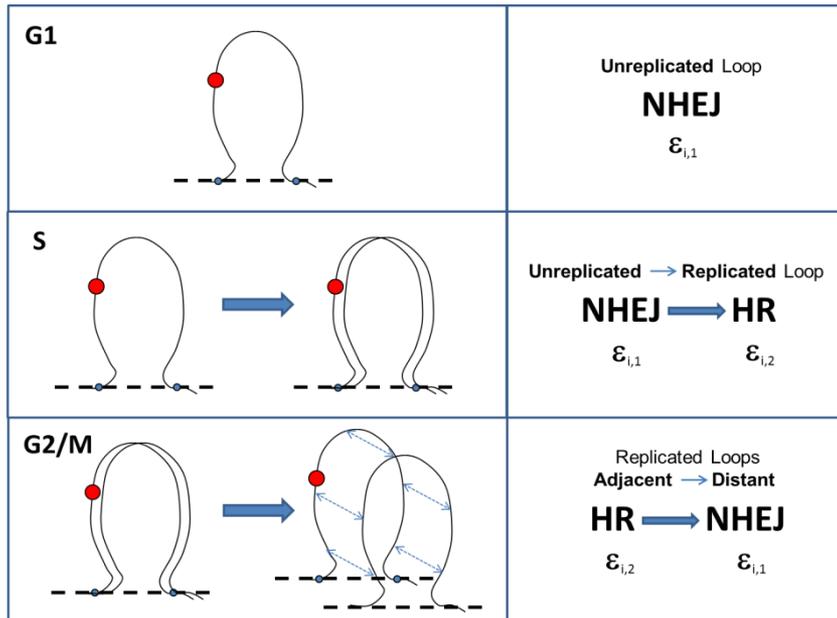


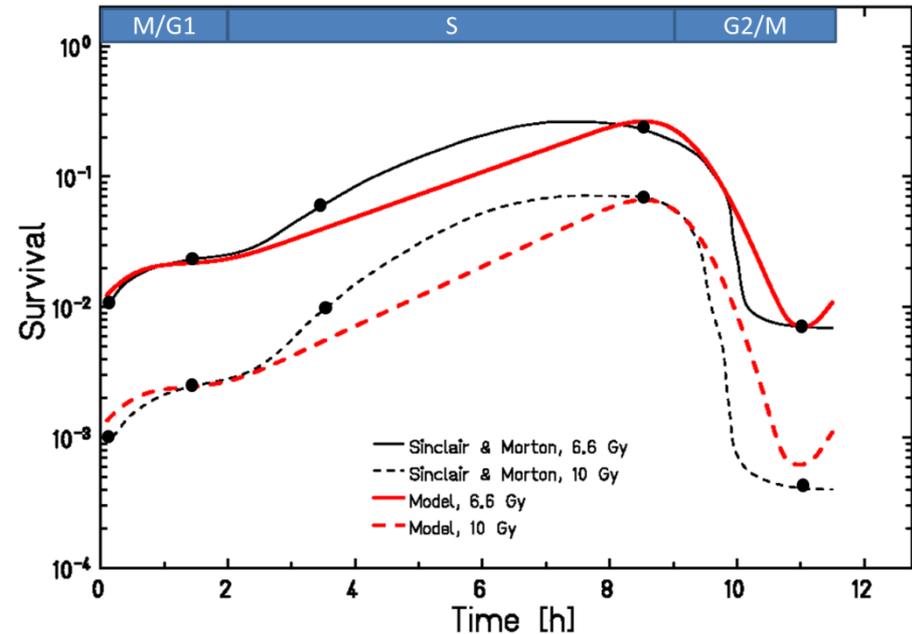**NHEJ**: Non-homologous end joining (error prone)
**HR**: Homologous recombination (error free)

$\varepsilon_{i,2} \sim 0$
$\varepsilon_{i,1} > \varepsilon_{i,2}$

$\varepsilon_{c} \gg \varepsilon_{i,1}$

Determine parameters from fits to
2 survival curves: G1-, late-S-phase

$\varepsilon_{i,1} = \mathbf{0.0179} \quad \pm 0.00063$
$\varepsilon_{i,2} = \mathbf{0.00054} \quad \pm 0.00087$
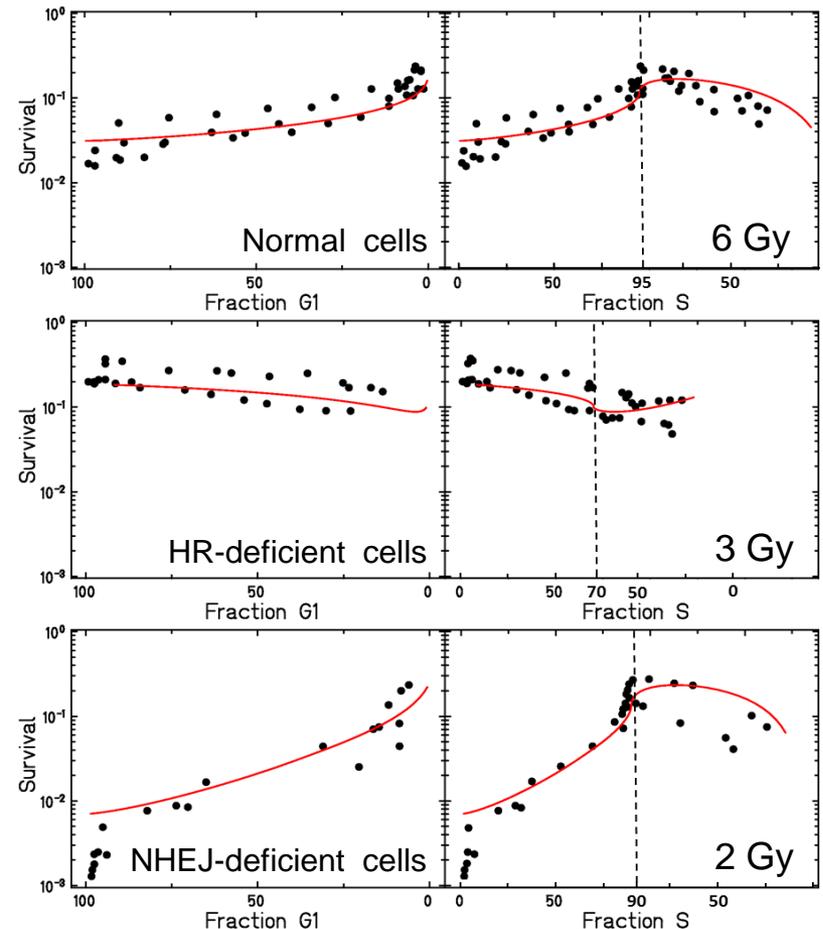$\varepsilon_{c} = \mathbf{0.0835} \quad \pm 0.011$



Hufnagl et al., DNA Repair 2015

# Cell cycle variation for repair deficient cells

Normal cells:

$\varepsilon_{i,1}=0.0179$    $\varepsilon_{i,2}=0.00054$    $\varepsilon_c= 0.0835$
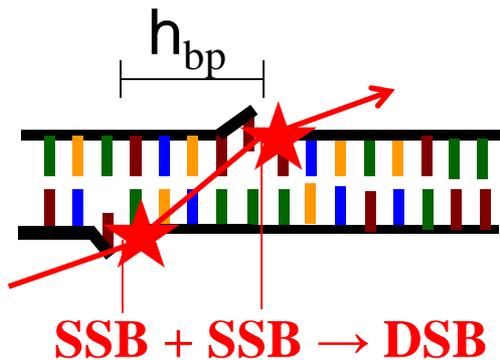
HR-deficient cells:
Repair of iDSB only by NHEJ

$\varepsilon_{i,1}=0.0179$    $\varepsilon_{i,2}=0.0179$    $\varepsilon_c= 0.0835$

NHEJ-deficient cells:
iDSB in unreplicated loops as lethal as cDSB

$\varepsilon_{i,1}=0.0835$    $\varepsilon_{i,2}=0.00054$    $\varepsilon_c= 0.0835$



- Exp. Data: Hinz et al., *DNA Repair 2005*
- Model: Hufnagl et al., *DNA Repair 2015*

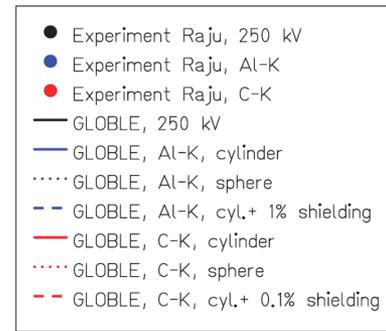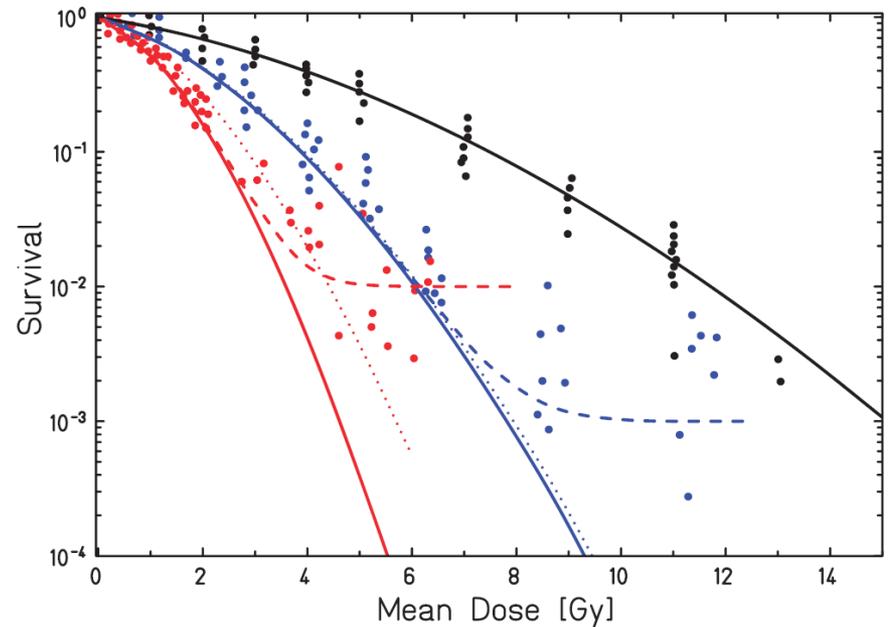$h_{bp}$

$$SSB + SSB \rightarrow DSB$$

Ultrasoft x-rays

⬇

Low-energy photo electrons
„Electron track ends"

⬇

higher yield of DSB

| Radiation quality | Energy (keV) | RBE$_{DSB}$ (from de Lara *et al.*) |
|---|---|---|
| C-K | 0.28 | 2.7 |
| Al-K | 1.49 | 1.9 |
| Ti-K | 4.55 | 1.4 |

But: random distribution



- ● Experiment Raju, 250 kV
- ● Experiment Raju, Al-K
- ● Experiment Raju, C-K
- —— GLOBLE, 250 kV
- —— GLOBLE, Al-K, cylinder
- ······ GLOBLE, Al-K, sphere
- – – GLOBLE, Al-K, cyl.+ 1% shielding
- —— GLOBLE, C-K, cylinder
- ······ GLOBLE, C-K, sphere
- – – GLOBLE, C-K, cyl.+ 0.1% shielding

Friedrich et al., Radiat. Res. 2014

# Conclusion and Outlook

Description of biological effects in terms of **spatial DSB pattern** represents a powerful tool to predict radiobiological effects after high- and low-LET irradiation

Spatial pattern is characterized with respect to giant-loop chromatin structure, which emphasizes the role of micrometer clustering

Distinction between isolated DSB (iDSB) and clustered DSB (cDSB)

Direct link to repair pathways, depending on replication status of loop

Ongoing work:

Dose rate / split dose effects after high-LET irradiation
Cell cycle variation of sensitivity after high-LET irradiation
Merge LEM & GLOBLE

# Thank you!