Multiscale modeling for the study of intra-cell dynamics following FLASH irradiation

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Outline

- ✓ Multi-scale modeling in nano-bio-physics and molecular biology
- ✓ FLASH effect: phenomenology and focus on the time and size scales
- ✓ Triggering steps: the ROS production
- ✓ Subsequent steps: to the macro-scale
- ✓ A 'holistic' approach: integration AI driven

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Radioterapie e diagnostiche avanzate in oncologia

Responsabile scientifico: Leonida Gizzi

SPOKE 1

SPOKE LEADER

· Consiglio Nazionale delle Ricerche (CNR)

Partner affiliati

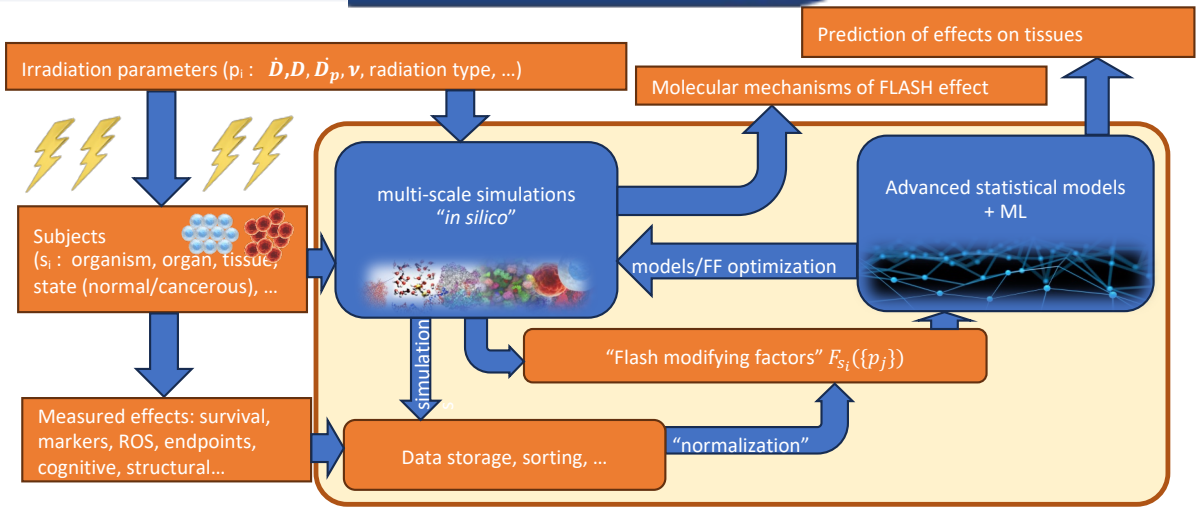
· Università di Firenze (UNIFI)

· Università di Pisa (UNIPI)

· Istituto Nazionale di Fisica Nucleare (INFN)

Sub-project 2

Molecular mechanisms, in vitro validation and radiobiological effect modelling of ultra-high dose rate



M1.2.1 Developed and implemented multi-scale procedure for the in silico simulation of the UHDR (FLASH-RT)

M1.2.2 Radiobiological effects at UHDR vs. CONV and radiation parameters

M1.2.3 Developed and implemented a database for sharing, elaborating and combining data from experiments and simulations

Multi-Scale modeling of bio-processes

Biological processes are inherently multi-scale

→ Multi-scale modeling is needed to simulate them

Multi-scale modeling

= Use different resolution/models/ representations for modeling the different stages of a process

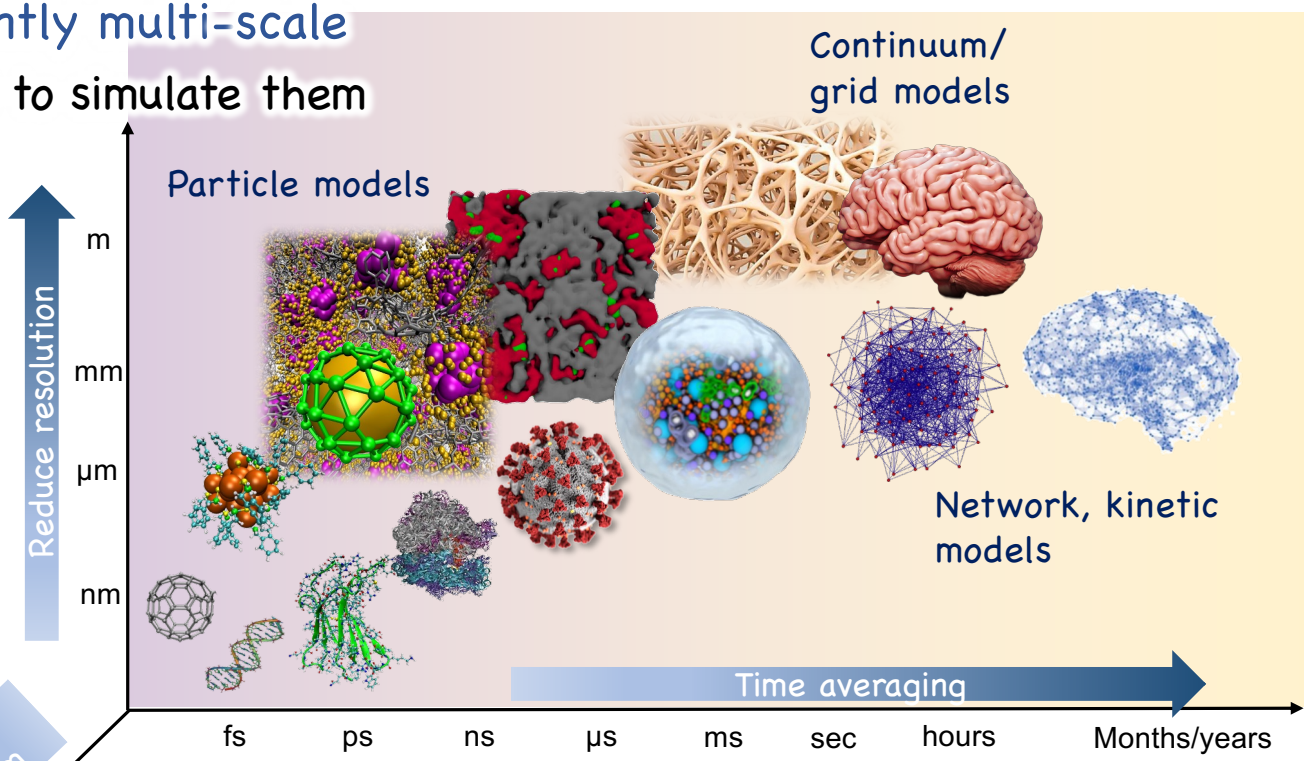
Spatial coarse graining

- ✓ Reduce/hide degrees of freedom
- ✓ Use collective variables

Parameters coarse graining

Interactions have increasing empiricism

- ✓ Strategy for fitting parameters (regression/ML)
- ✓ accuracy/trasferability
- ✓ Target/input data



Time coarse graining

- ✓ Increase the time step of motion integration
- ✓ cut off high frequencies
- ✓ Use stochastic/enhanced sampling methods, statistical averages

Multi-Scale modeling of bio-processes

Spatial coarse graining

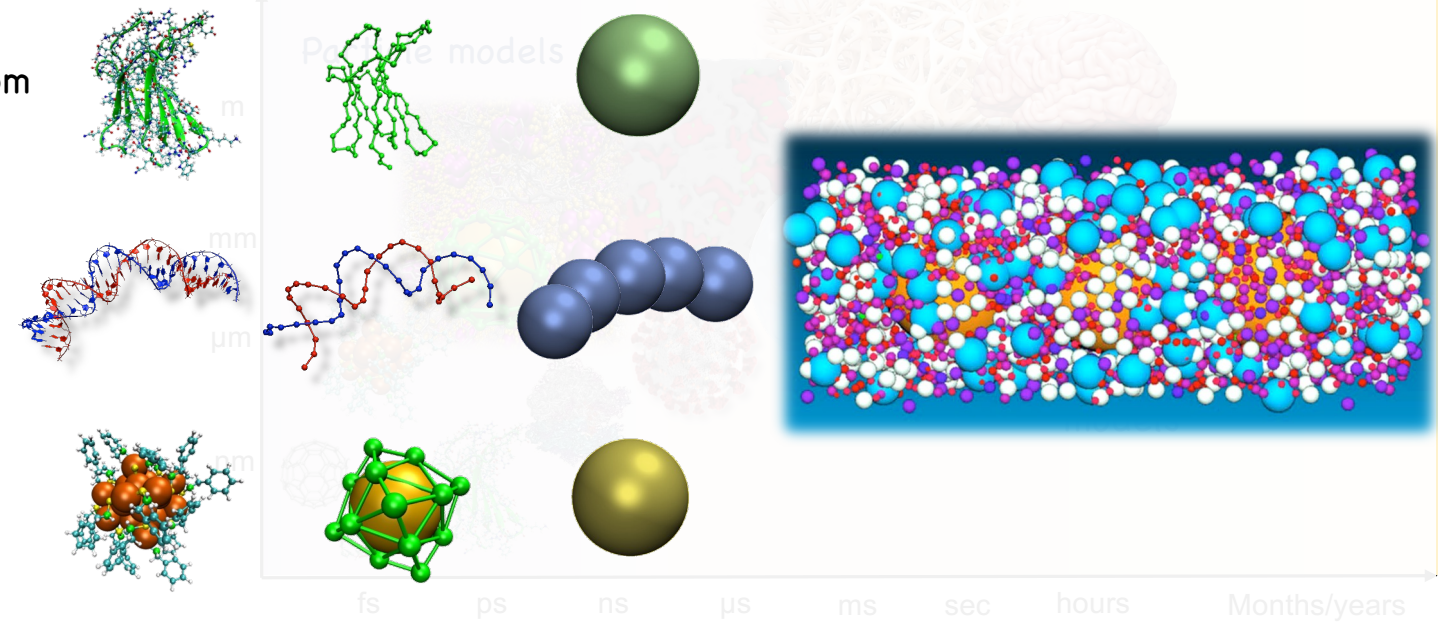
- ✓ Reduce/hide degrees of freedom
- ✓ Use collective variables

Reduce resolution

Time coarse graining

- ✓ Increase the time step of motion integration
- ✓ cut off high frequencies
- ✓ Use stochastic/enhanced sampling methods, statistical averages

Time averaging



"Parameters" coarse graining

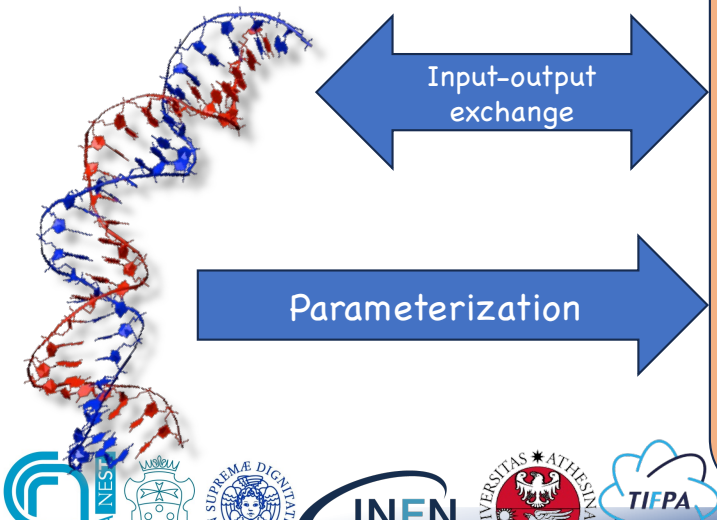
Interactions have increasing empiricism

- ✓ Strategy for fitting parameters (regression/ML)
- ✓ accuracy/trasferability
- ✓ Target/input data

Increase empiricism

Multi-Scale modeling of bio-processes

- ✓ The multi-scale approach involves the feedback between different stages AND with experiment
- ✓ Simulations and calculations return observables to compare with experiment
- ✓ Experiment gives feedback to models and aid parameterization
- ✓ Simulations give prediction for quantities unaccessible to experiment
- ✓ **Muli-metodological integration is necessary**



Input-output exchange

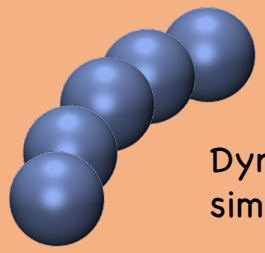
Parameterization

Predictions

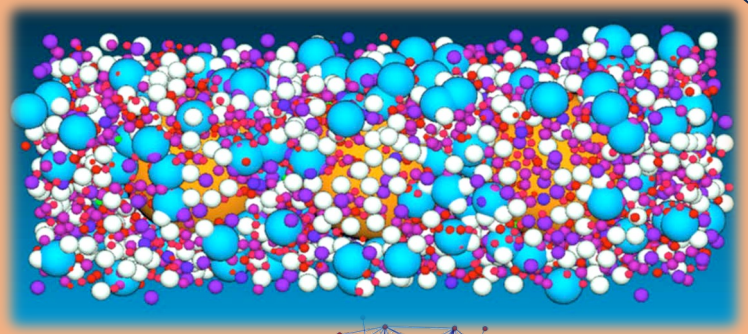
Expt data

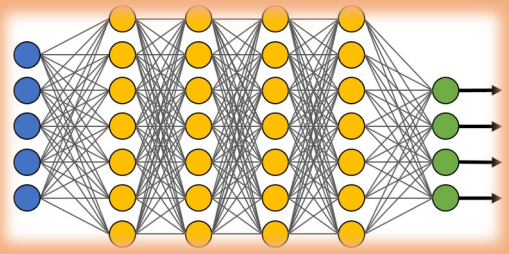
params refinement

Observable calculation

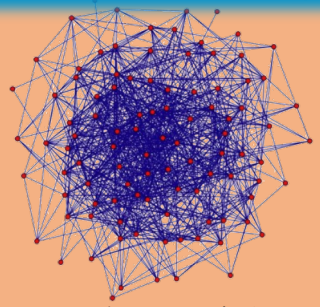


Dynamics simulations



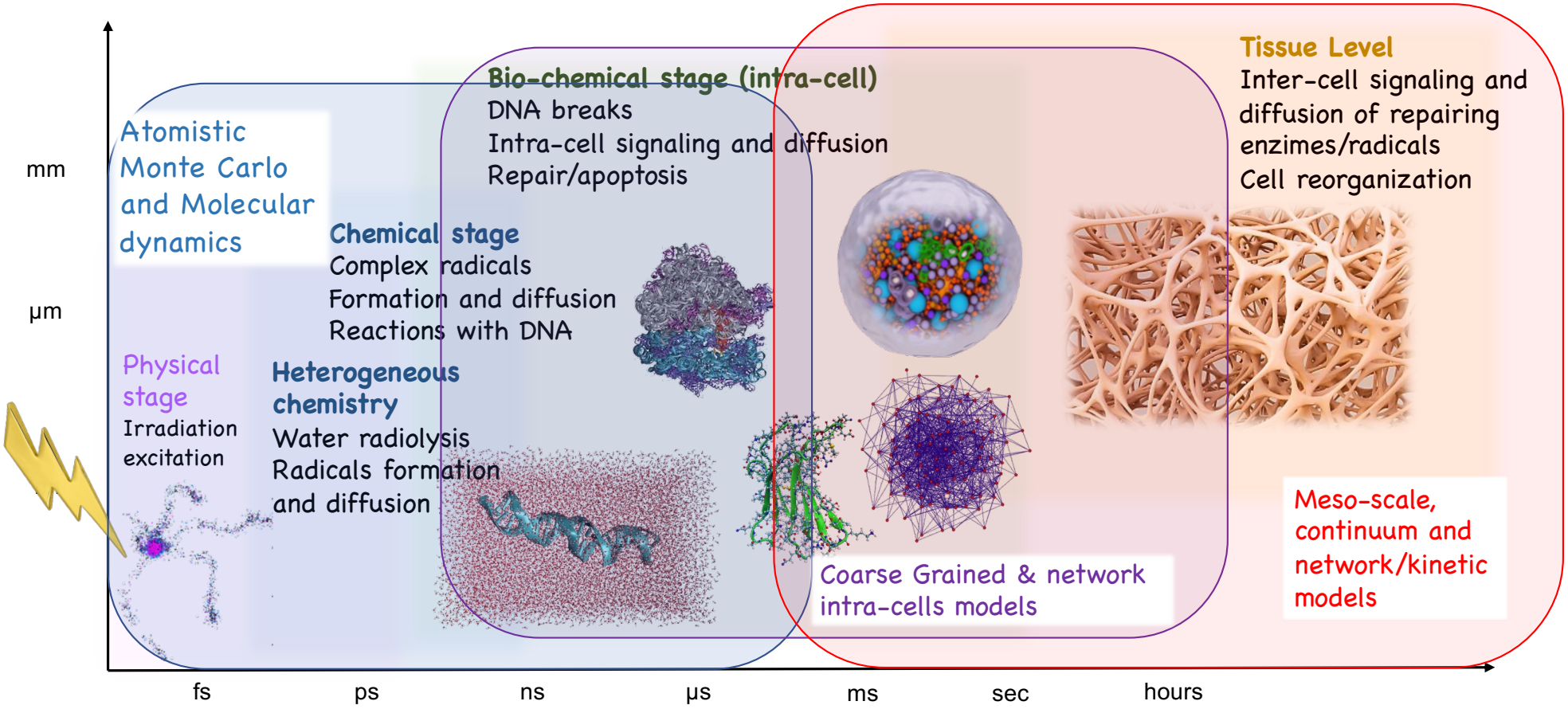


Adnvanced fit procedures, ML



Kinetic models

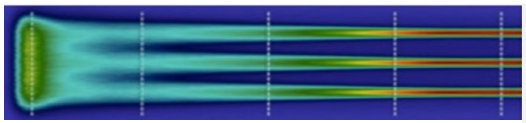
Multi-scale modeling radiation damage



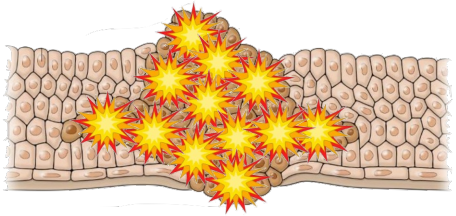
Time and space dynamics of FLASH

Minibeam irradiation

mm-μm comb



How does FLASH effect act?



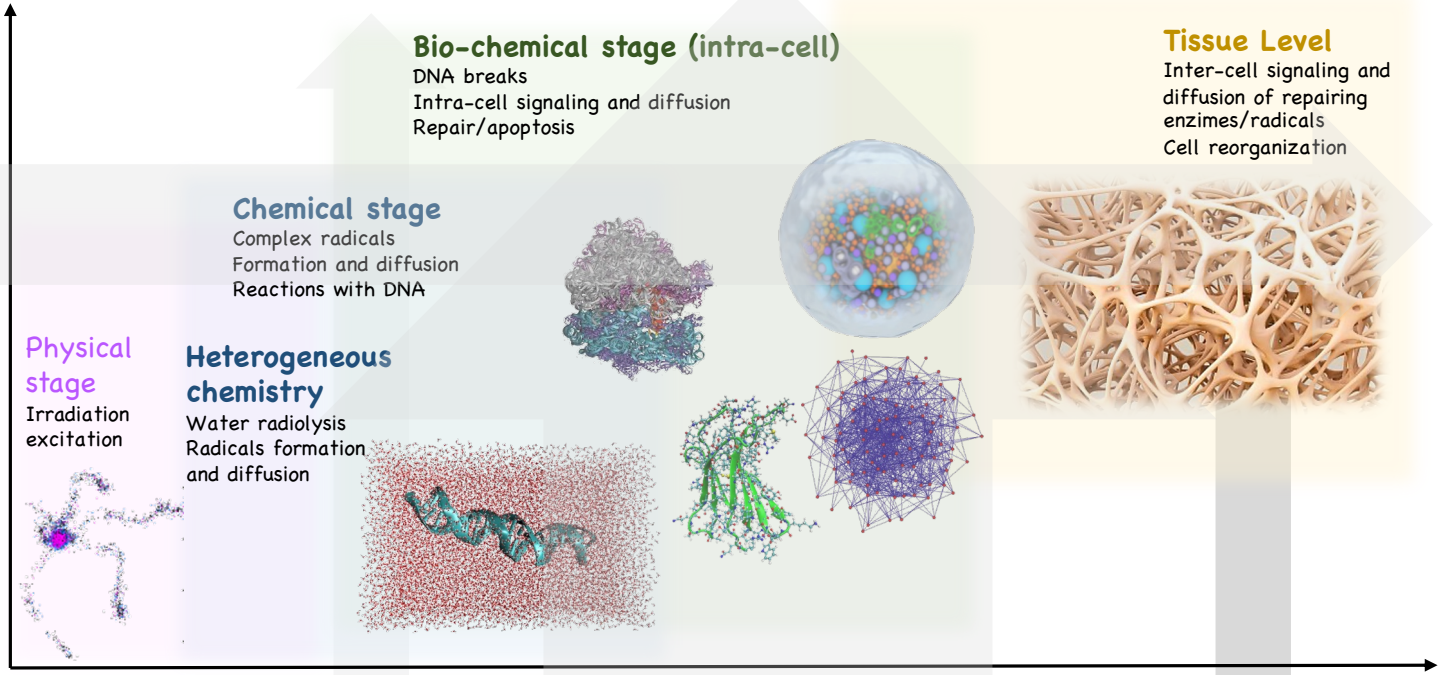
Physical stage
Irradiation excitation

Chemical stage
Complex radicals
Formation and diffusion
Reactions with DNA

Heterogeneous chemistry
Water radiolysis
Radicals formation and diffusion

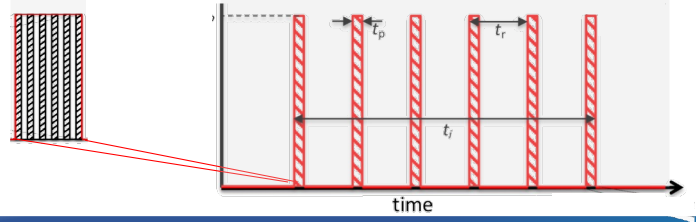
Bio-chemical stage (intra-cell)
DNA breaks
Intra-cell signaling and diffusion
Repair/apoptosis

Tissue Level
Inter-cell signaling and diffusion of repairing enzimes/radicals
Cell reorganization



Flash irradiation features

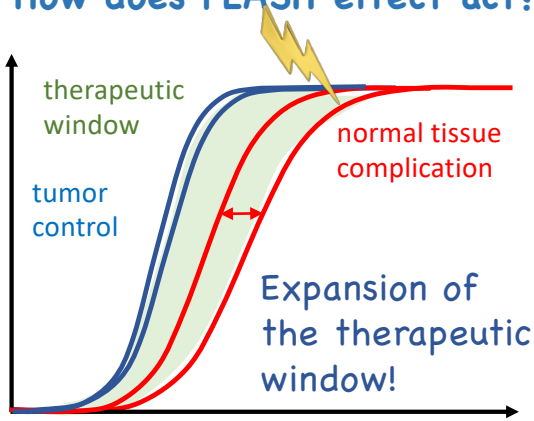
- ✓ Very high dose rate ~10^6 Gy/sec
- ✓ Pulsed irradiation
  - pulse duration μsec
  - pulse period 1-100 msec
- ✓ Intrapulse GHz (psec-nsec scale)



Differential cell survival/ tissue sparing

Phenomenology and definitions

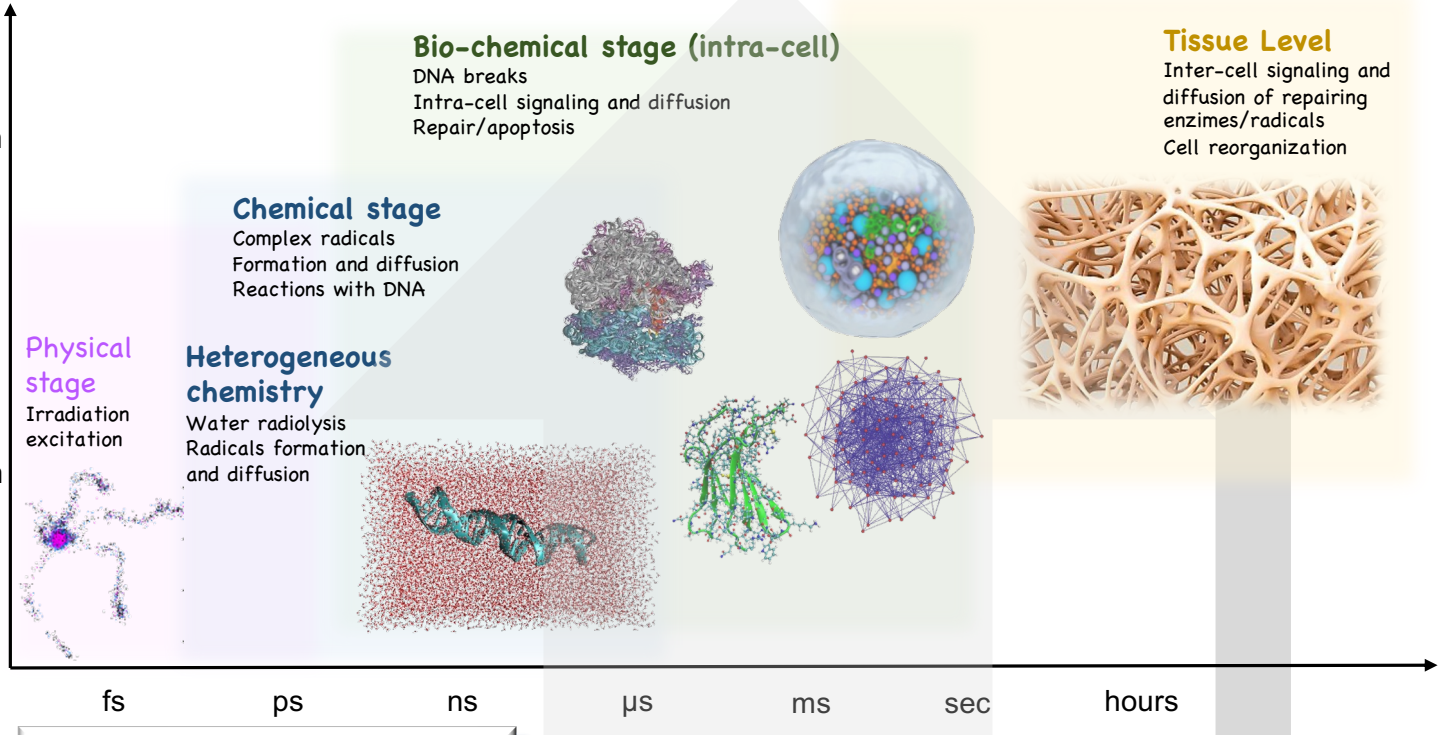
How does FLASH effect act?



Quantification

Modifying factor

$$MF = \frac{D_{FLASH}}{D_{CONV}}\bigg|_{isoeffect}$$



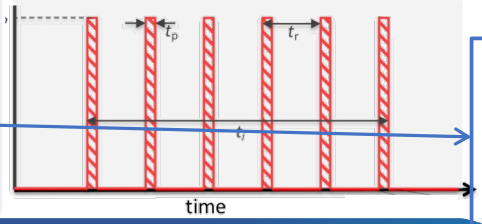
MF depends on the radiation params

$MF = MF(D, \dot{D}, t_p, t_r, \ldots)$

and on the cell type/state

$MF = \mathrm{MF}_{\mathrm{cancer/healthy}}(D, \dot{D}, t_p, t_r, \ldots)$

Modeling aim:
Understand mechanicistic determinants of FLASH to better quantify the dependencies of MF



MF_{cancer}~1 and $MF_{healthy}$>1

Selective killing!

Time and space dynamics of FLASH

Why does Flash act differentially on healthy/cancer cells?

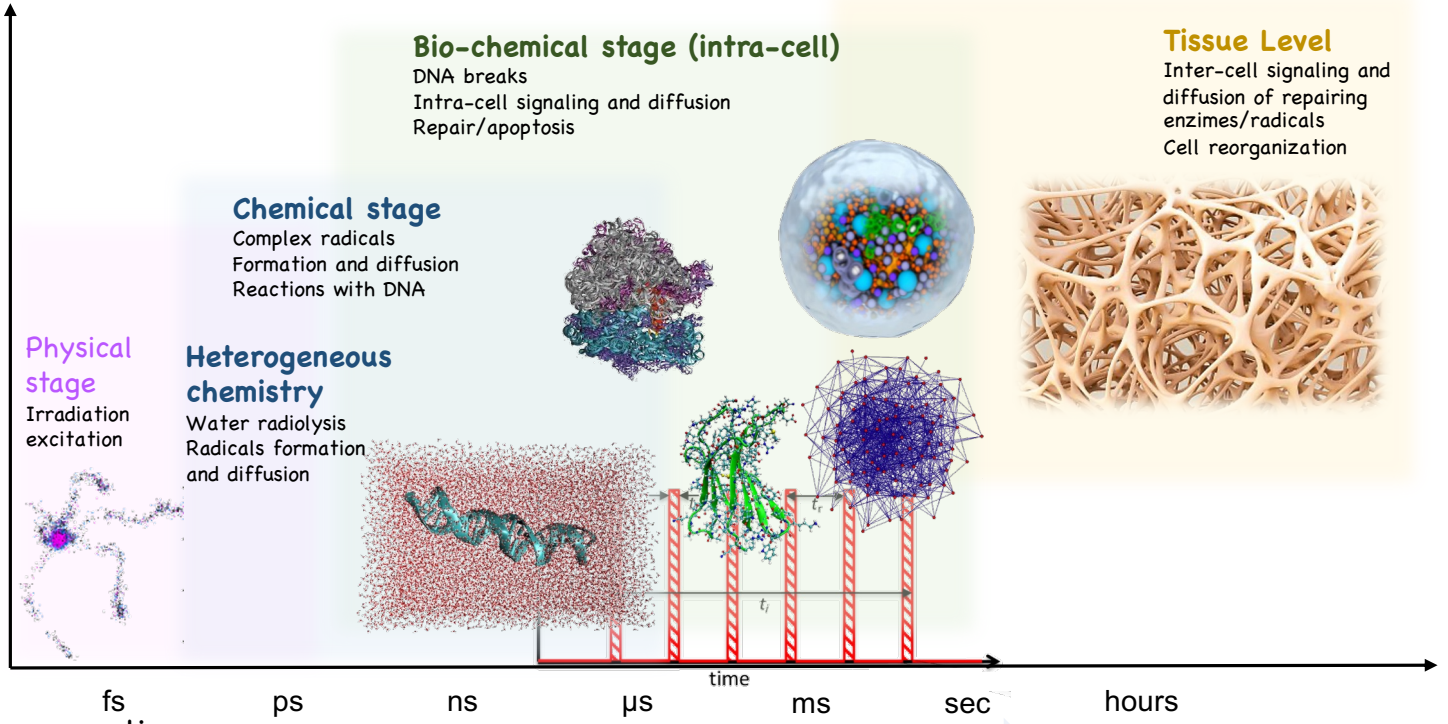
Possible determinants of differential effects

Oxygen concentration
⇒ Different production of ROO*

ROO* differential diffusivity
⇒ Reach different saturation level

Activation and diffusion of reparatory enzymes/mols
⇒ Different efficiency of DNA damage reparation

Activation of inter-cell signaling
⇒ Different damage propagation/remediation
→ survival/death



At different stages, different makers of the effects could be observed → **Feedback with expt**

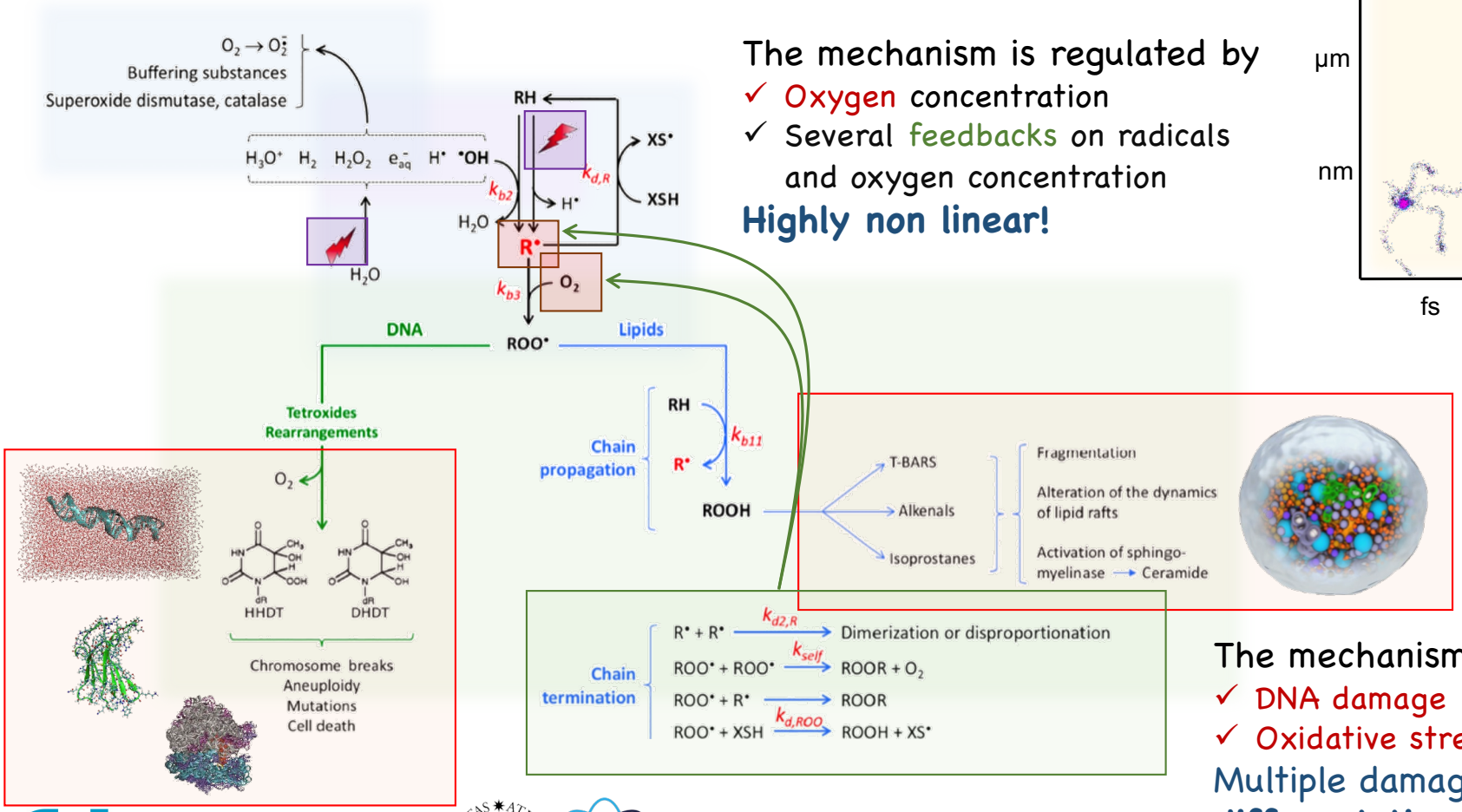
Oxygen concentration

ROO* diffusion/saturation

reparation enzymes & mechanisms

Cell signaling/death/survival

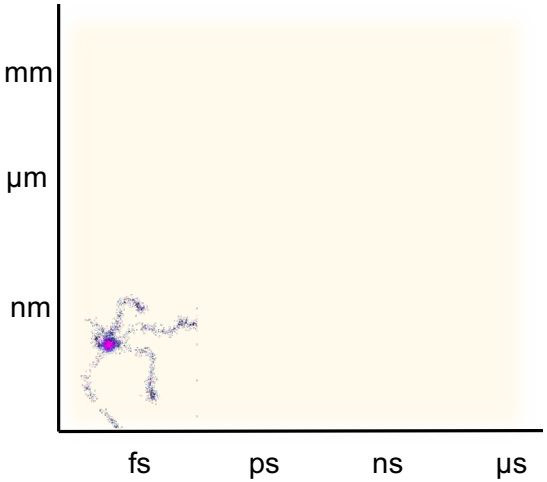
Radicals evolution scheme



The mechanism is regulated by

- ✓ Oxygen concentration
- ✓ Several feedbacks on radicals and oxygen concentration

Highly non linear!

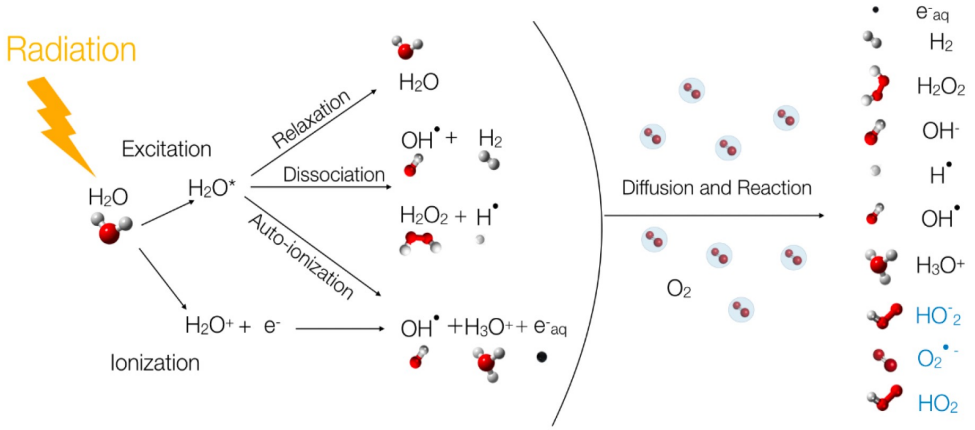


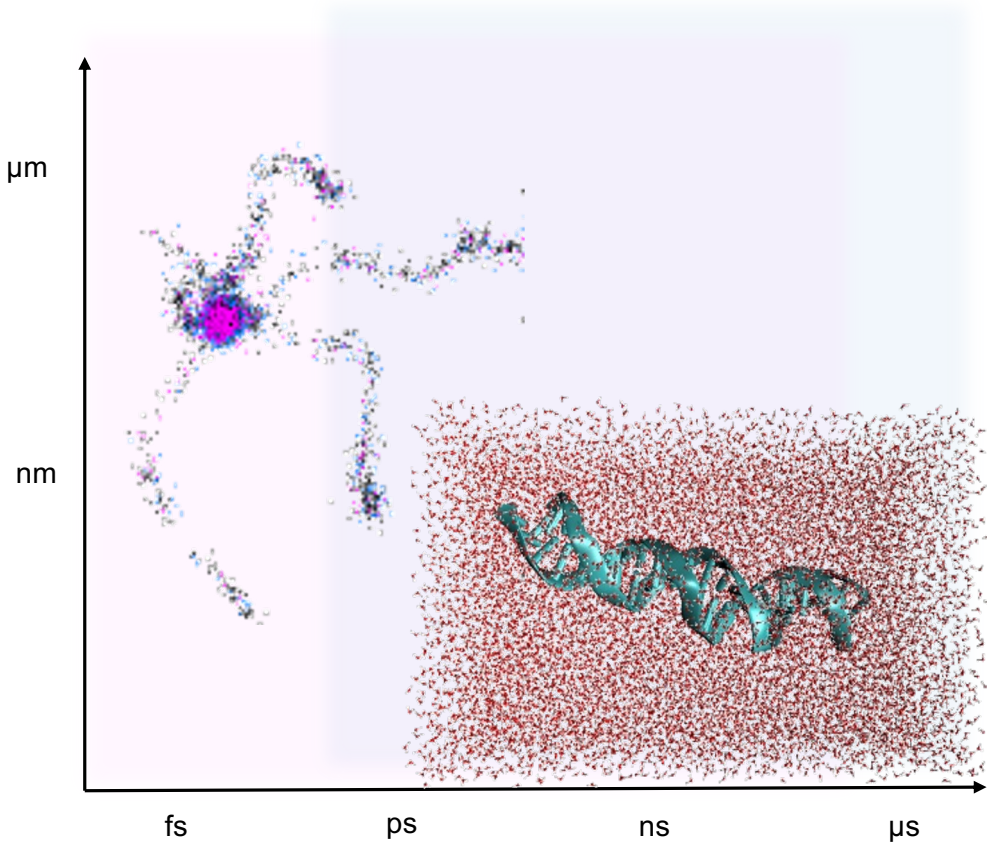
The mechanism generates

- ✓ DNA damage
- ✓ Oxidative stress

Multiple damage pathways, **different time scales**

Starting from the beginning… First steps of ROS generation





- ✓ **Computer simulation** of water radiolysis and formation early evolution/diffusion of ROS and interaction with biomolecules
- ✓ **Time-size scales:** up to μsec and μm
- ✓ **Method:** combine two "complementary" approaches

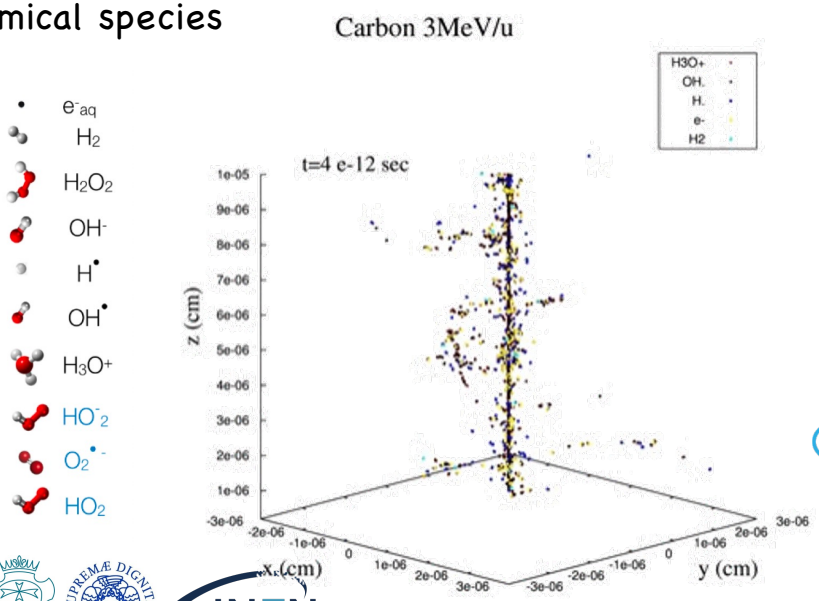
Reaction-diffusion Monte Carlo

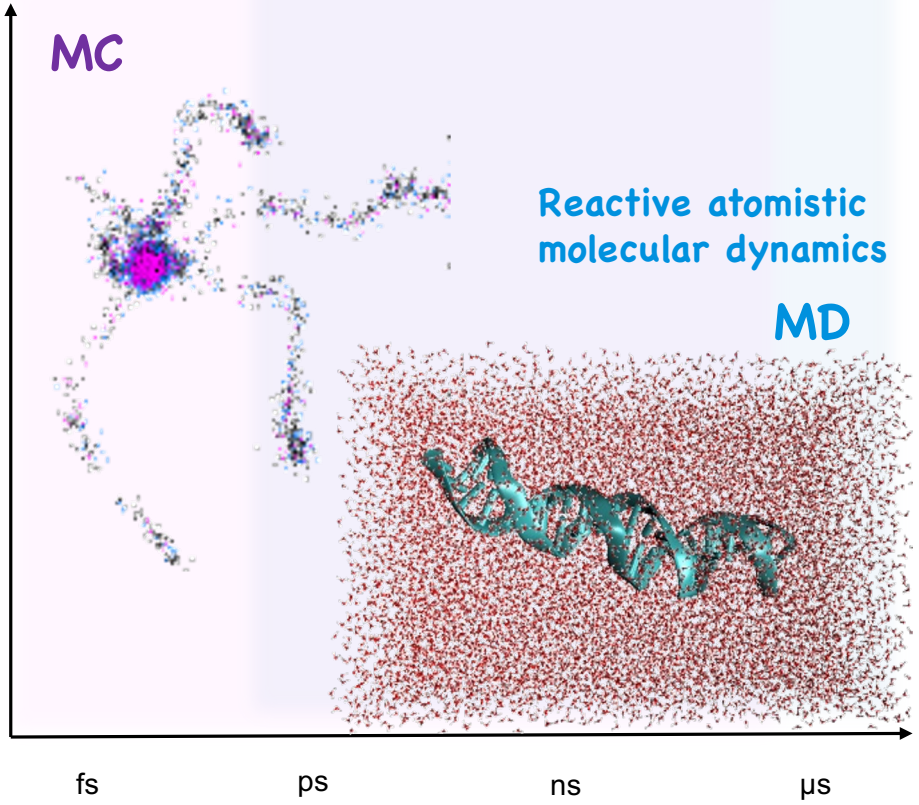
Reactive atomistic molecular dynamics

Combining Monte Carlo & Molecular dynamics

Reaction-diffusion Monte Carlo (RDMC)

- ✓ **Simulation procedure:** **Probability driven** (Metropolis, MC) **track based**
- ✓ **Input:** Cross sections, kinetic constants & diffusion coefficients; radiation type, energy and quality
- ✓ **Time/space scales:** fs to μs, nm to μm
- ✓ **Output:** tracks and spatial distribution in time of chemical species



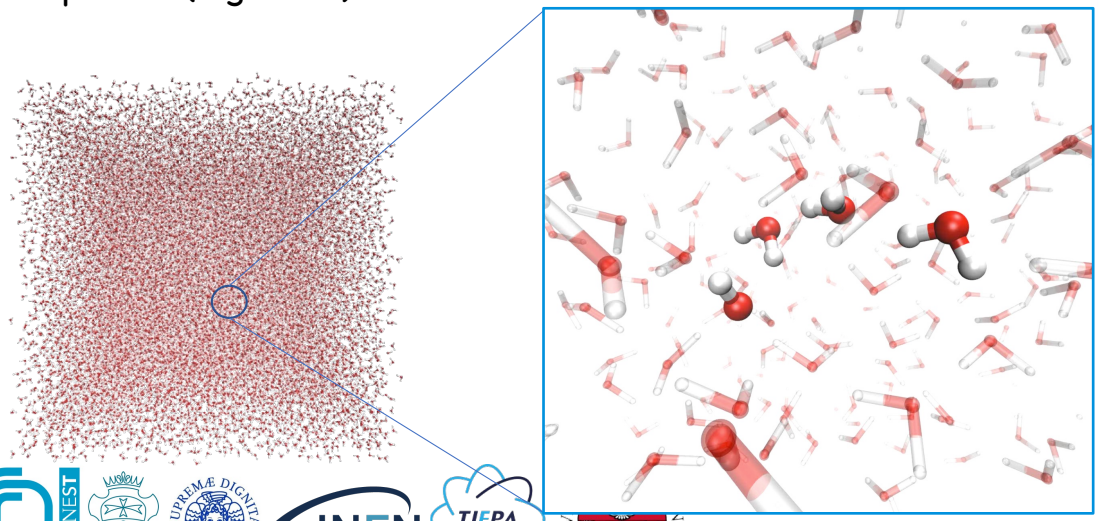


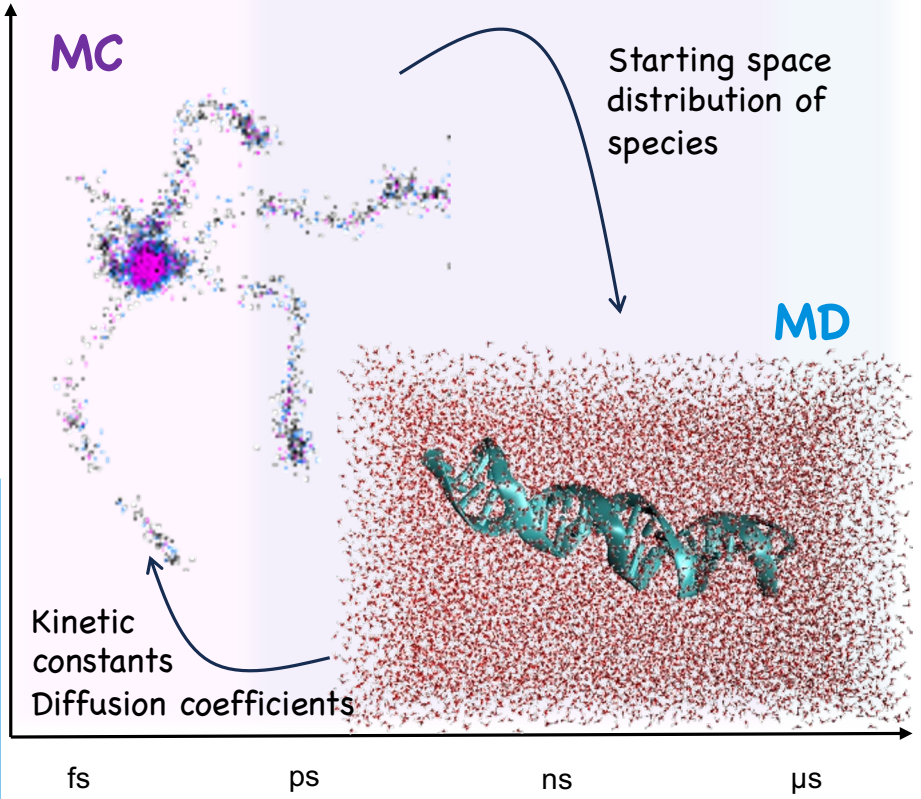
Boscolo, Scifoni et al 2018-2024
Castelli, Petrolli, Micheloni 2020-2024

Combining Monte Carlo & Molecular dynamics

Reactive atomistic molecular dynamics (RAMD)

- ✓ **Integration procedure:** reactive MD FFs **force driven** $M_i\ddot{\mathbf{R}}_i = -\nabla_i U(\{\mathbf{R}_i\}) - M_i\gamma_i\dot{\mathbf{R}}_i - \mathbf{F}_i^s$ **trajectory based**
- ✓ **Input:** Interactions $U(\{\mathbf{R}_i\})$, box of water molecules, + other molecules of interest in starting state
- ✓ **Time/space scales:** fs to μs, nm to μm
- ✓ **Output:** **Trajectories of each particle,** reactions evolution and diffusion, **kinetic constants** for new species (e.g. DNA)

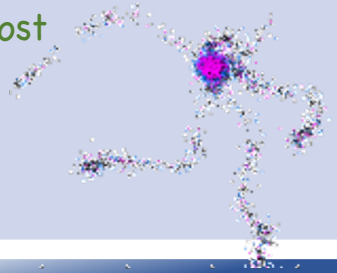


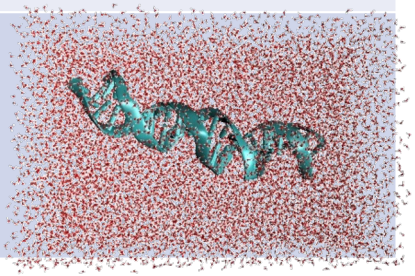


MC and MD are complementary!

MC and MD are complementary!

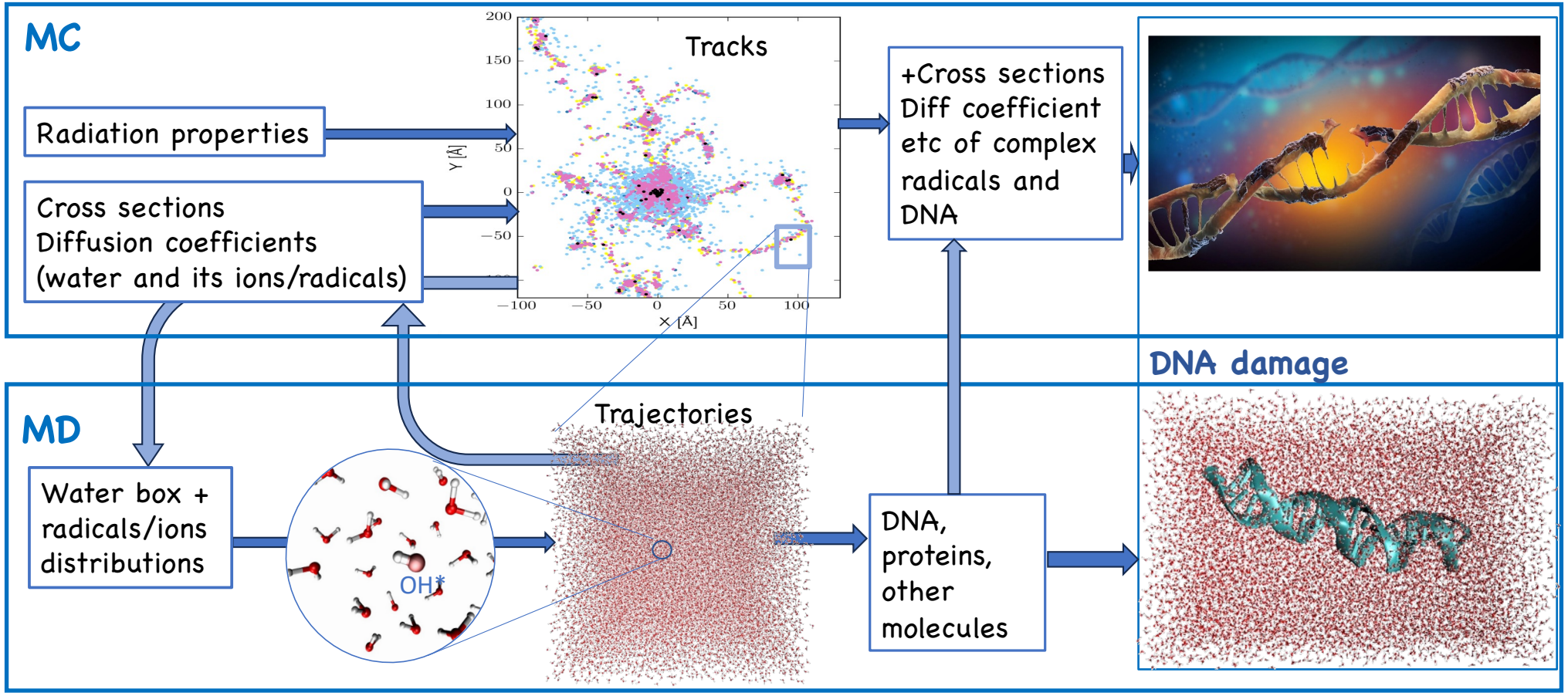
	Monte Carlo	Molecular Dynamics
Algorithm	Probabilistic, energy based	Deterministic, Force based
Degrees of freedom	Track based, the chemical evolution and diffusion of reactive species is followed in time	Trajectory based, dynamics of all the particles position and charge state is followed in time, chemical evolution is automatically included
timestep	fs to ps	fs
simulation length/size	microsec-millisec micron to mm	nanosec to microsec (millisec for coarse grained mdls) Nanometer to micron (subcell size, coarse grained mdls)
Simulation cost	Dependent on the number of species included in the simulation	Scaling polinomially with the number of DoS (~$N^{2-1.5}$), and therefore with the size of the system. Independent from the species included.
Empirical level	The diffusion coefficients, reaction cross sections are required as inputs for each of the reaction included	The Hamiltonian is required as input, adding different species in the system comes at no additional effort
	Lower computational cost Larger empirical level	Lower empirical level Larger computational cost





MC and MD are complementary!

Interfacing MC with MD

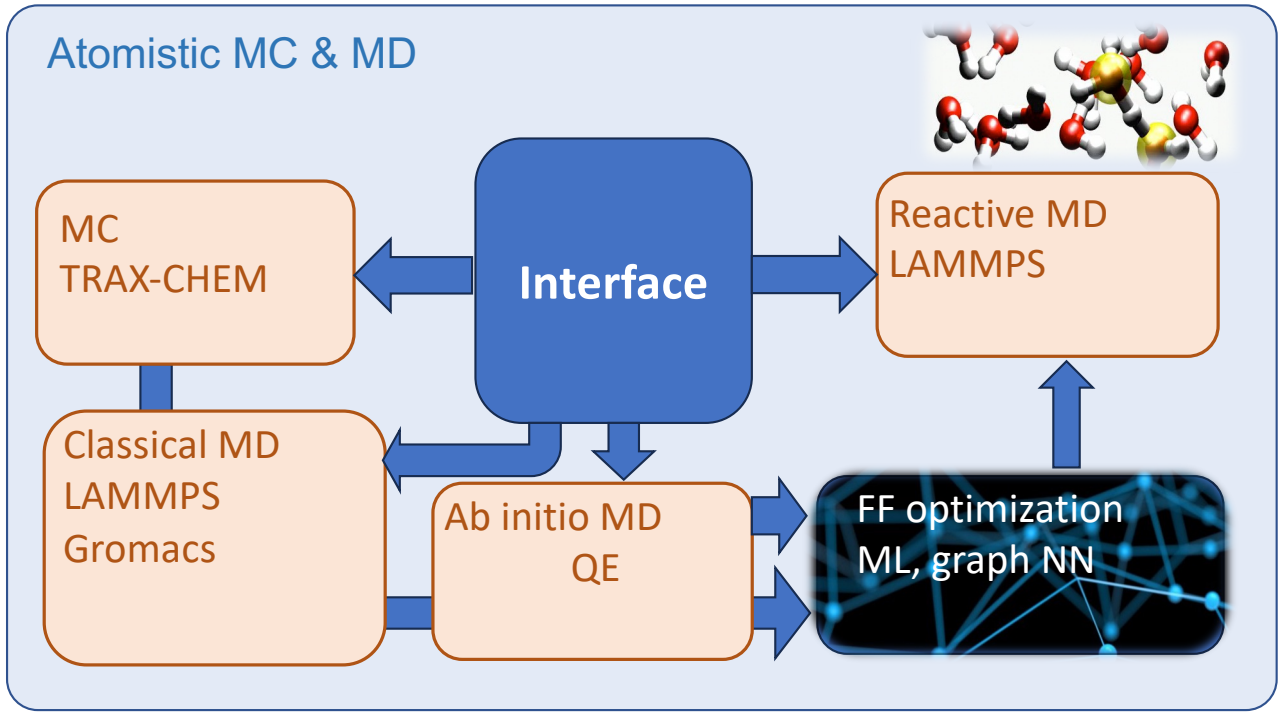


Interfacing MC with MD

MC and **MD** are implemented in different codes (TRAX-CHEM and LAMMPS)

An interface is currently under construction to:

(Lorenzo Castelli, Emanuele scifoni, Lorenzo Petrolli, Manuel Micheloni)



- ✓ Exchange configuration/trajectory data
- ✓ Calculate cross sections and other parameters from MD to give in input to MC
- ✓ Calculate particles distribution from MC to give in input to MD
- ✓ Use other kind of more fundamental MD (e.g. ab initio) to optimize the interactions in reactive MD

These tasks are aided by **NN and ML algorithms**

Example benchmark: water radiolysis

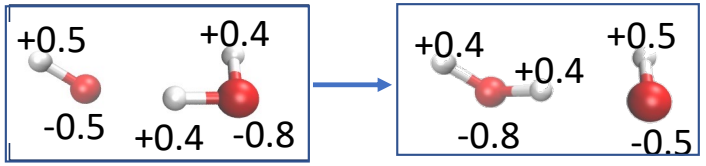
Example benchmark: water radiolysis

L Castelli, L Petrolli, E Scifoni, V Tozzini

How does the reactive FF works with the radical species?

$$M_i\ddot{\mathbf{R}}_i = -\nabla_i U(\{\boldsymbol{R}_i\})$$

✓ **Forces** to evolve atomic species are calculated from a unique **interaction potential accounting for reactivity** (formation and breacking of bonds)

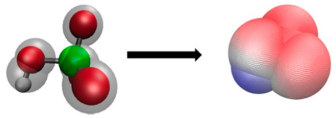


✓ **Charges on each atom/ion/radical must dinamically evolved** at each time step (fractional charge account for bonds polarization

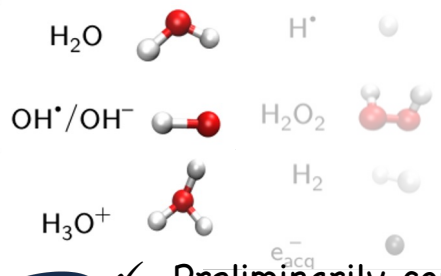
⇒ **An accurate method to evolve the charge is essential!**

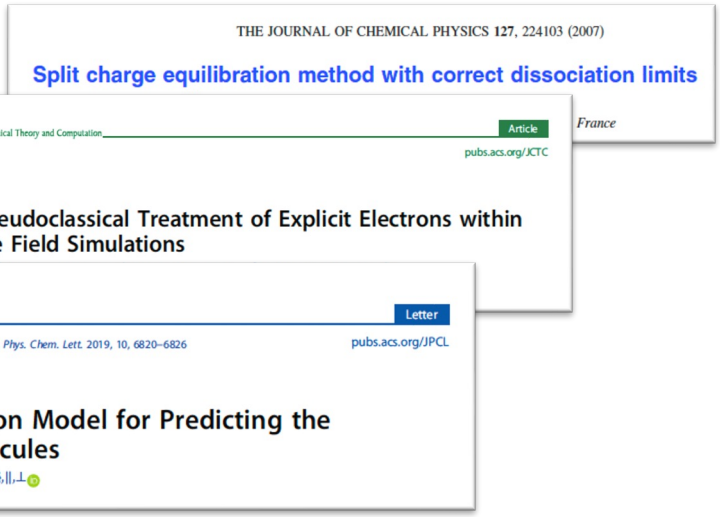
✓ Three different methods:

- ReaxFF **Qeq** (Electrochemical potential equilibration)
- ReaxFF **ACKS2**
- **CGeM**



Species	D ($10^{-9}m^2s^{-1}$)
$OH^•$	2.8
H_3O^+	9.0
$H^•$	7.0
e^-_{aq}	4.5
H_2	4.8
OH^-	5.0
H_2O_2	2.3
O_2	2.1
$HO_2^•$	2.0
HO_2^-	2.0
$O_2^{•-}$	2.1





✓ Preliminarily consider a **reduced number of chemical species**

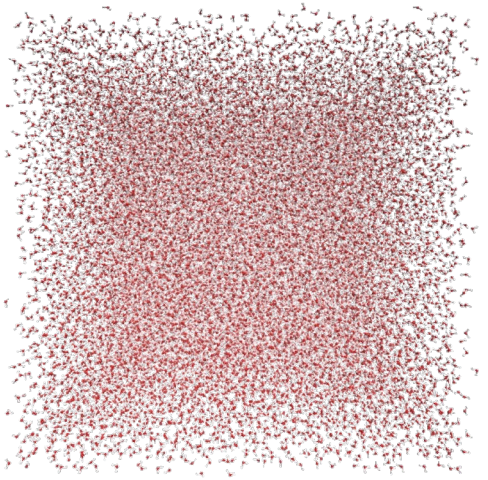
Water radiolysis

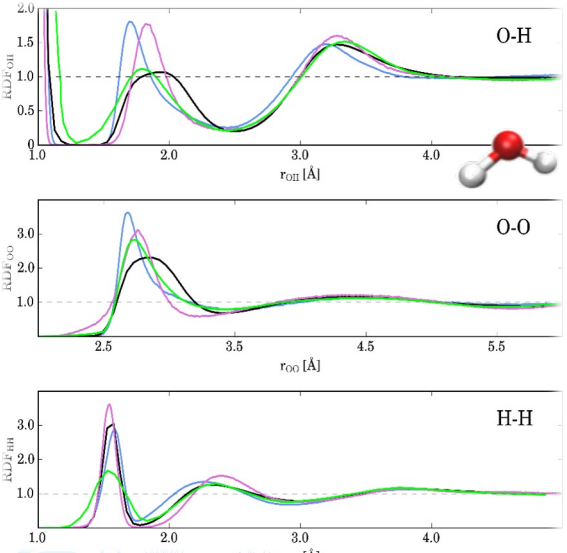
H_2O 

$OH^{\bullet}/OH^{-}$ 

H_3O^{+} 

- ✓ Preliminarily consider a reduced number of chemical species
- ✓ Create a **water box** (10-100 nm)
- ✓ Run **long simulations** (μsec overall) in at room conditions
- ✓ Calculate the **pair distribution functions** and **diffusion coefficients**



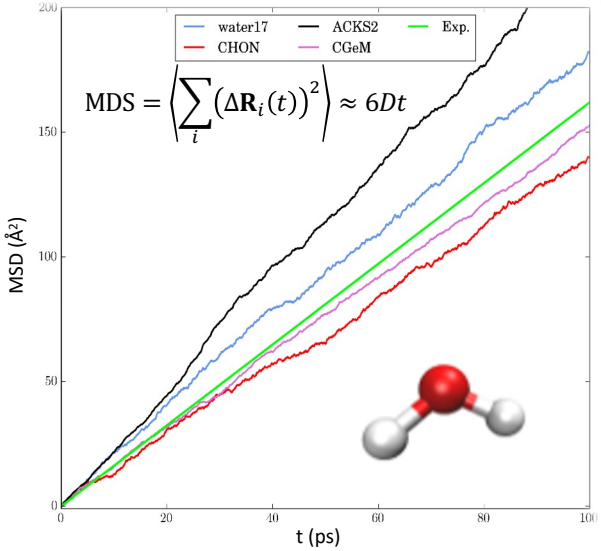


The structure of water

radial distribution functions g(r) (probability of finding particles at distance r) are similar in the three methods and compare fairly well with the experimental data

The water diffusivity

is better reproduced by CGeM, generally overestimated by the other methods

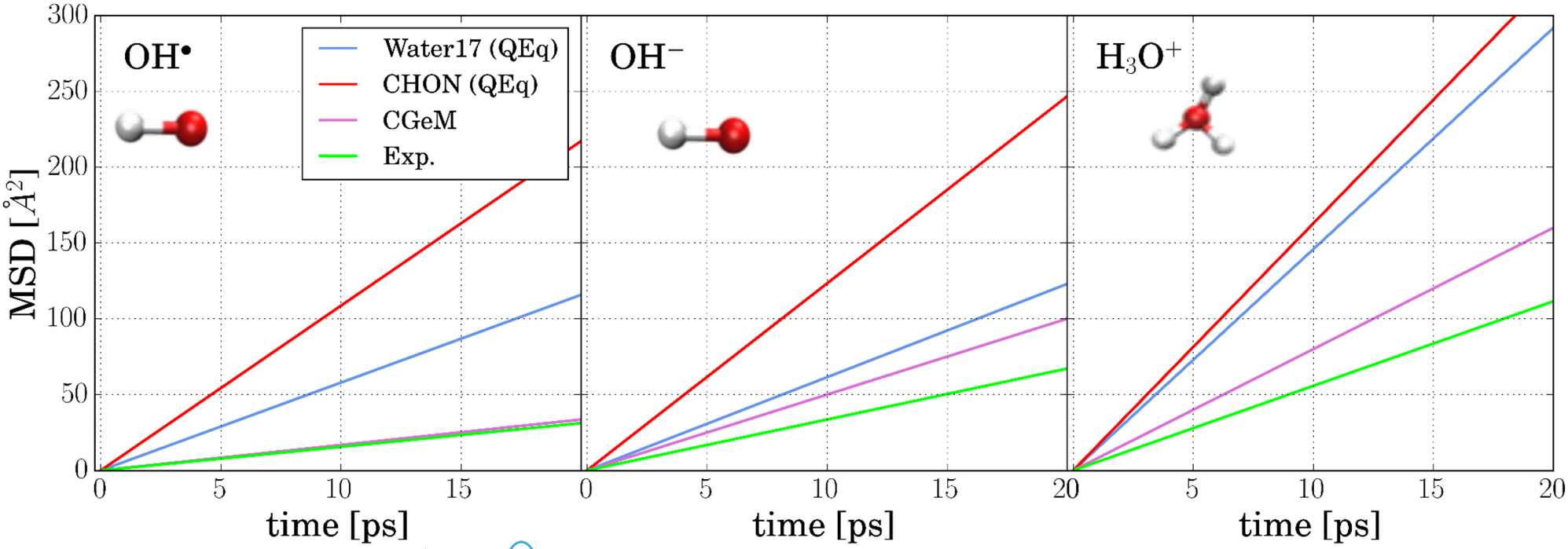


Ions and radical diffusion

Diffusion coefficient is less good

- ✓ In **reasonable agreement** only with CGeM
- ✓ Largely overestimated with **other methods**

Why?

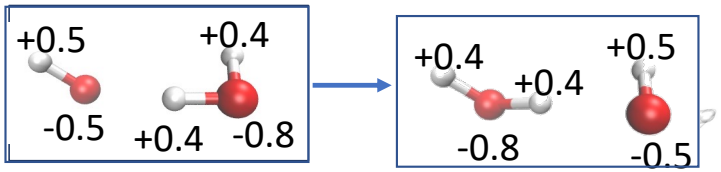


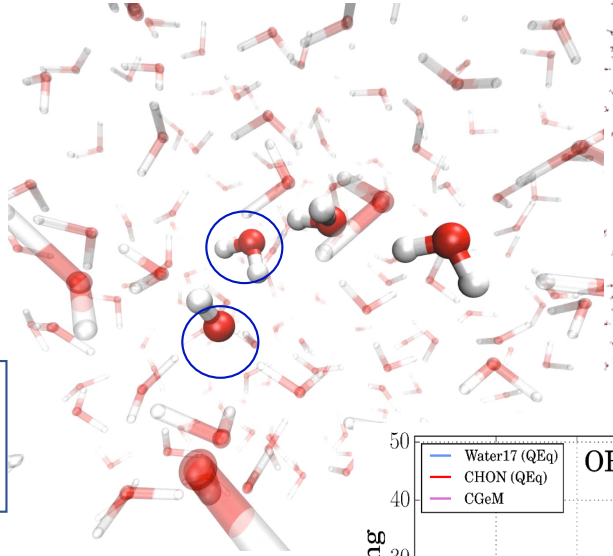
Ions and radical diffusion

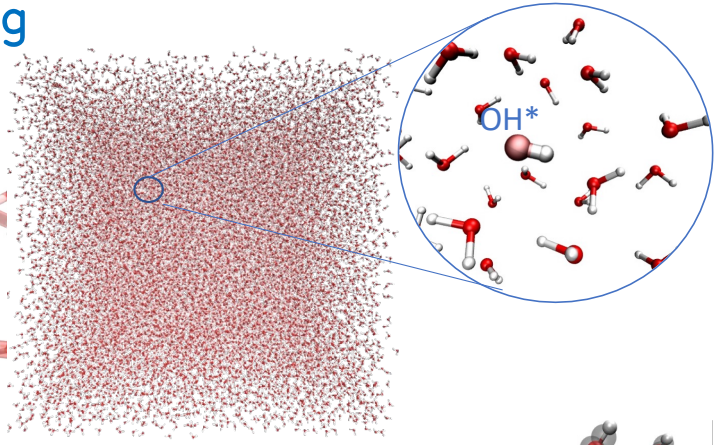
OH* and OH⁻ diffusion is correlated with proton hopping

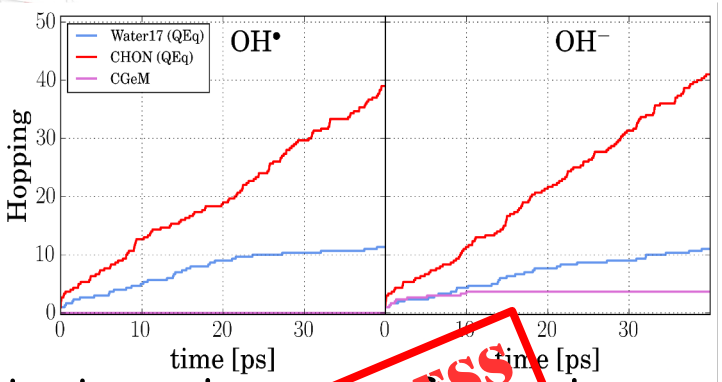
$$OH^* + H_2O \rightarrow H_2O + OH^*$$
$$OH^- + H_2O \rightarrow H_2O + OH^-$$

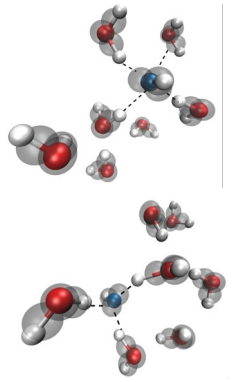
The hopping probability is related to the charge dynamics









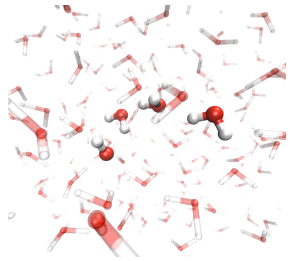


- Qeq produces too fast and fractional charge transfer ⇒ **too fast hopping**
- Qeq is not able to distinguish ions and radicals
- Missing discrete nature of the electronic charge!

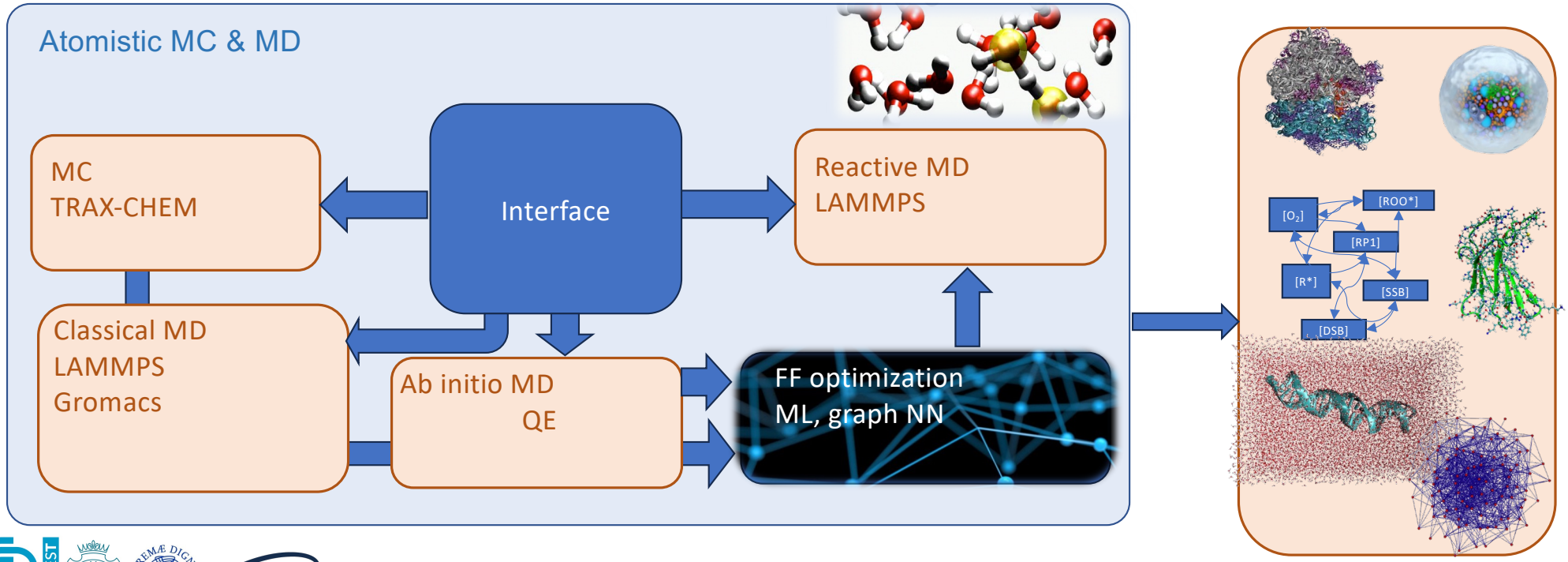
CGeM reincludes explicit electron degrees of freedom and and correct charge hopping dynamics!

IN PROGRESS

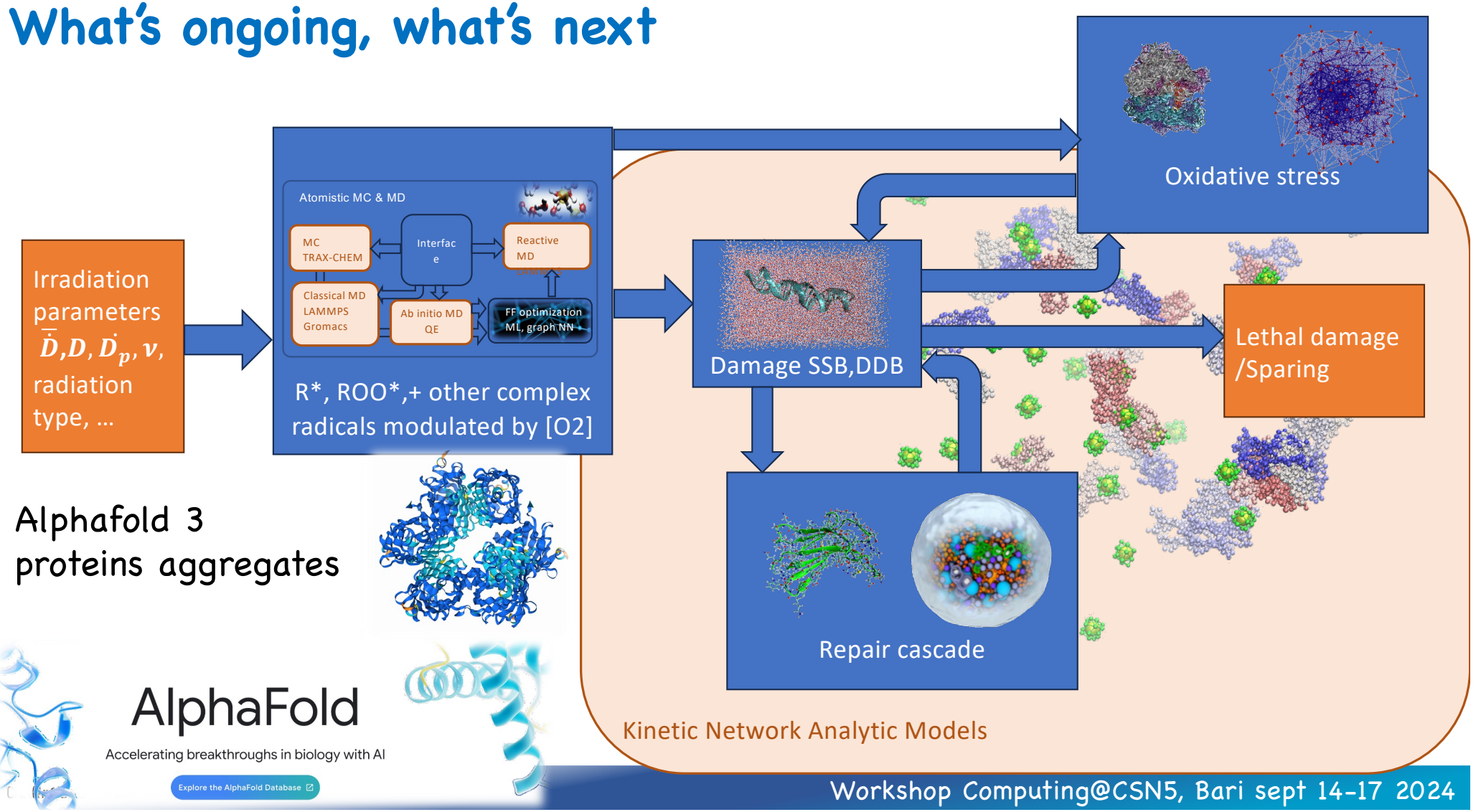
What's ongoing, what's next



- ✓ The "atomistic" simulations must be included in a more complete platform including the other stages of simulations



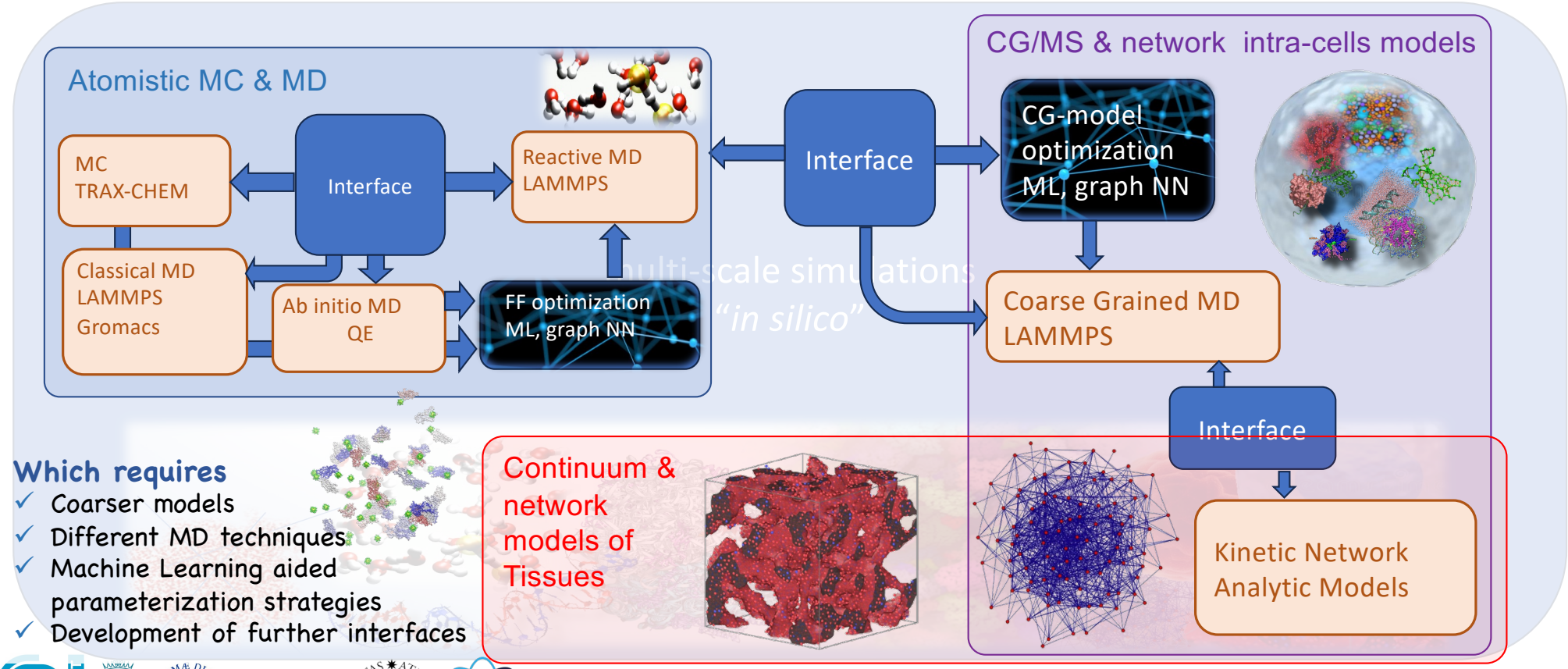
What's ongoing, what's next



Alphafold 3
proteins aggregates

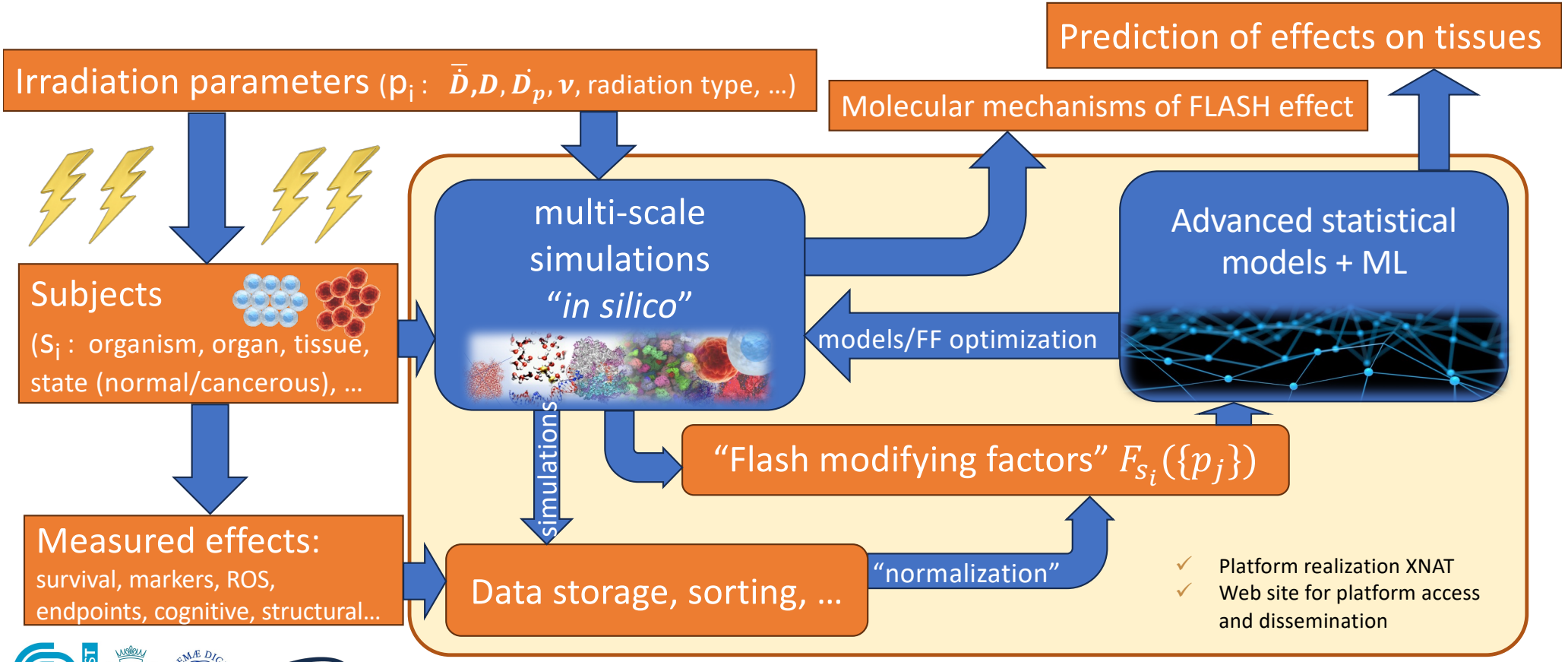
What's ongoing, what's next

✓ The interface is a part of the multi-scale simulation platform...



What's ongoing, what's next

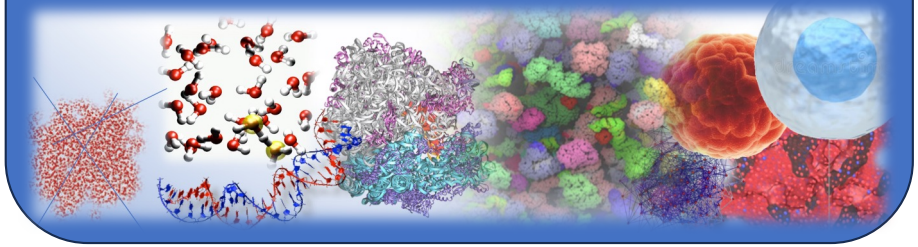
… which is part of a general analysis and modeling platform of **FLASH effect**…



Summary

- ✓ **Computer modeling** is a valuable tool to investigate the mechanistic determinants of the FLASH effect, but **multi-scale methods** must be used
- ✓ A **comprehensive platform** is under construction to combine the different methods and explore the different possible determinants, from the role of oxigen to that of differential damage repair mechanism
- ✓ At the atomistic level, **Monte Carlo** and **Molecular Dynamics** are complementary methods and must be combined to explore the physical and chemical phases of the radiobiological cascade up to the micro-scale
- ✓ The larger scales (sub-cell and intra-cell biochemical cascade) must be explored with **coarse grained models** and **kinetic network** models
- ✓ The tasks of analysis, interfacing and parameter optimization and **effect prediction** are aided by **machine learning** techniques

multi-scale simulations
"in silico"



Thank you for your attention … AND aknowledgements

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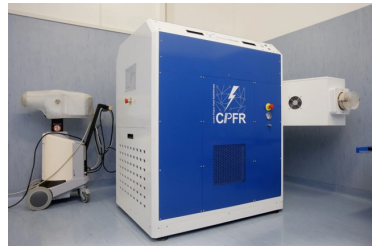

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