I. RAPID KINETIC METHODS

WHAT DETERMINES THE RATE OF A COMPLEX BIOCHEMICAL REACTION?

The elementary steps of a reaction

\[ P \xrightarrow{k_1} P^* \xrightarrow{k_2} PL^* \]

When/why shall we use rapid kinetic method?

RAPID KINETIC METHODS

Flow techniques
- Two solutions rapidly and completely mixed in a "mixing chamber"
- Observation "downstream" from the chamber
- Aging of the solution "dead time"

Relaxation techniques
- System at equilibrium is perturbed by applying a rapid change in some external parameter
- The system's response is the suitable optical signal

MOST IMPORTANT TECHNIQUES

Flow techniques
- Stopped flow
- Quenched flow
- Continuous flow

Relaxation techniques
- Temperature jump
- Pressure jump
- Flash photolysis
**STOPPED FLOW**
- Low amount of sample needed
- Dead time: typically 0.5-2 ms
- Plunger hits a block

**QUENCHED FLOW**
- Different „aging time“
- Q: quencher; stops the reaction (e.g.: acid, EDTA/EGTA (metal chelators))
- Series of quenched reactions—kinetics of the reaction determined

**CONTINUOUS FLOW**
- The optical signal is monitored at different points downstream from the mixer, and translated into the time-dependent signal change on the basis of the flow rate
- SF was developed from this method
- Faster (dead time: around 0.1 ms)
- Efficiency can be greatly improved using CCD
- SF has better sample economy
- CF not able to measure kinetics out to long times (would be expensive)

**TEMPERATURE JUMP**
- Joule heating: the temperature increase resulting from the resistance to an electric current flowing through it
- Temperature jumps: produced by the discharge of a high voltage capacitor
- Up to 5°C in 1-2 ms
- Laser heating: 10 ns

**PRESSURE JUMP**
- Piezoelectric crystal: generates large pressure increases (up to 200 atm)
- Small sample volume, fast reaction (50 ms)
- Long time scales; high ionic strength not required
- p induced perturbations are smaller than the T induced ones
- Intrinsic fluorescence signals show little pressure sensitivity

**FLASH PHOTOLYSIS**
- The reaction is triggered by a pulse of light
- „Caged compounds“: release the active ligand species to enable the application of a particular ligand at or near its site of action
- Works in milliseconds (or faster) time scale

Caged compounds:
- Caged nucleotides (e.g. ATP)
- Caged forms of neuroexcitatory amino acids (e.g. L-glutamate)
- Caged calcium (to study the Ca signal of the cell)

II. SURFACE PLASMON RESONANCE
Plasmon
- plasma: oscillation of mobile electrons at the surface of the metal film
- the oscillating plasma waves are called surface plasmons

Total internal reflection

The intensity of reflected light reaches minimum "dip"

WHAT SPR IS GOOD FOR
- To study recombinant proteins
- even without any labeling or modification
- in real time
- even in case of low affinity
- investigate the effect of mutations on the properties of interaction between molecules

WHAT SPR IS NOT GOOD FOR
- High throughput analysis
- Studying small analytes (Mr~1000 Da)

Thank you for your attention! Enjoy your meal!