REFEYN
WEIGHING MOLECULES WITH LIGHT

Mass Photometry: The new gold standard for biophysical characterization

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Regional Sales Manager

Biophysical characterization: key questions

Research & Discovery

Understanding biomolecular interactions and behaviors

Process Development

Optimizing the product and process

Quality Control

Assessing sample purity and quality

Biophysical characterization in product development

Research & Discovery | Process Development | Quality Control

Understanding biomolecular interactions and behaviours

- What are the compositions of the interacting species?
- Does the expected complex form & what is its stoichiometry?
- How does the complex assemble?
- How stable is the complex & how strong are the interactions?

Biophysical characterization in product development

Research & Discovery | **Process Development** | Quality Control

Optimizing products and processes

- How stable is the formulation?
- What conditions give the optimal product performance,
- What conditions are best for producing the desired protein (or other product)?

Biophysical characterization in product development

Research & Discovery | Process Development | **Quality Control**

Assessing sample purity and quality

- Is the sample in the required state to progress to the next stage of analysis / development / production?
- Does the product meet its critical quality attribute requirements?
- What is the AAV empty/full ratio? - GMP compliant soon!

Why using mass photometry?

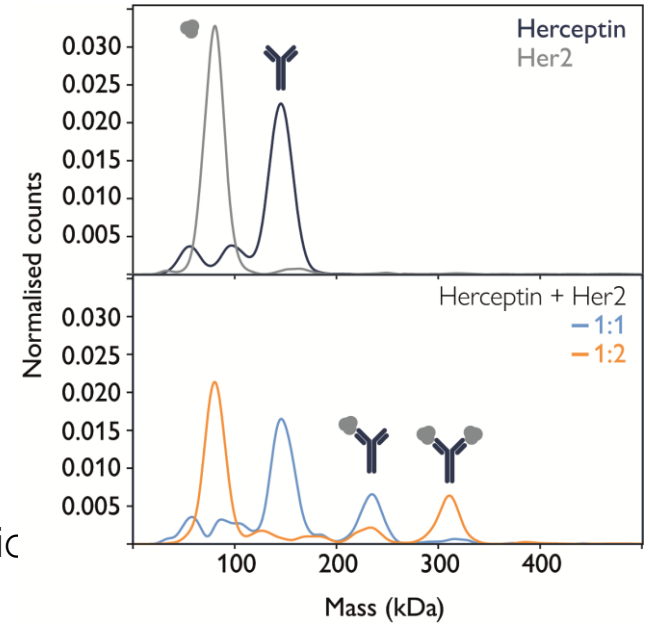
An innovative biophysical analysis tool with numerous applications

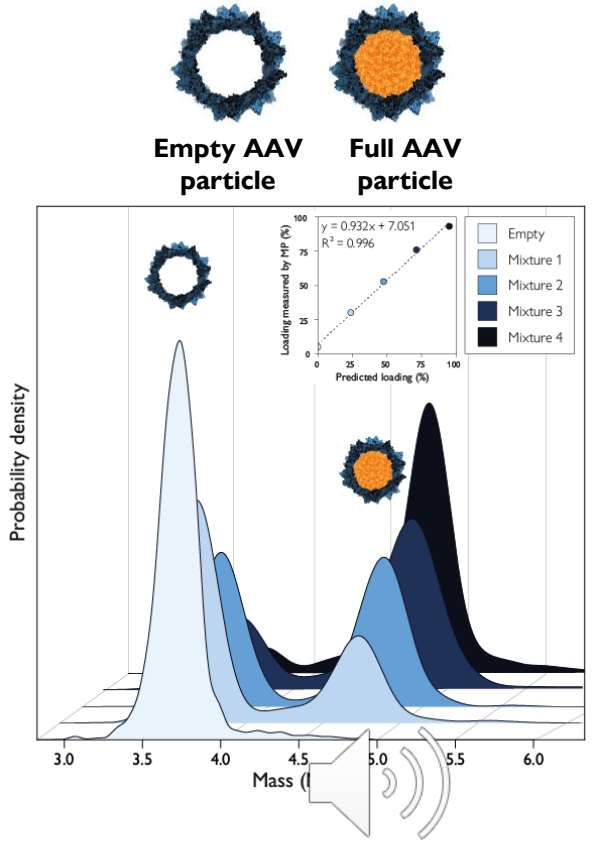
Mass photometry delivers

rapid, accurate mass measurement of

label-free single molecules in

solution in their native state

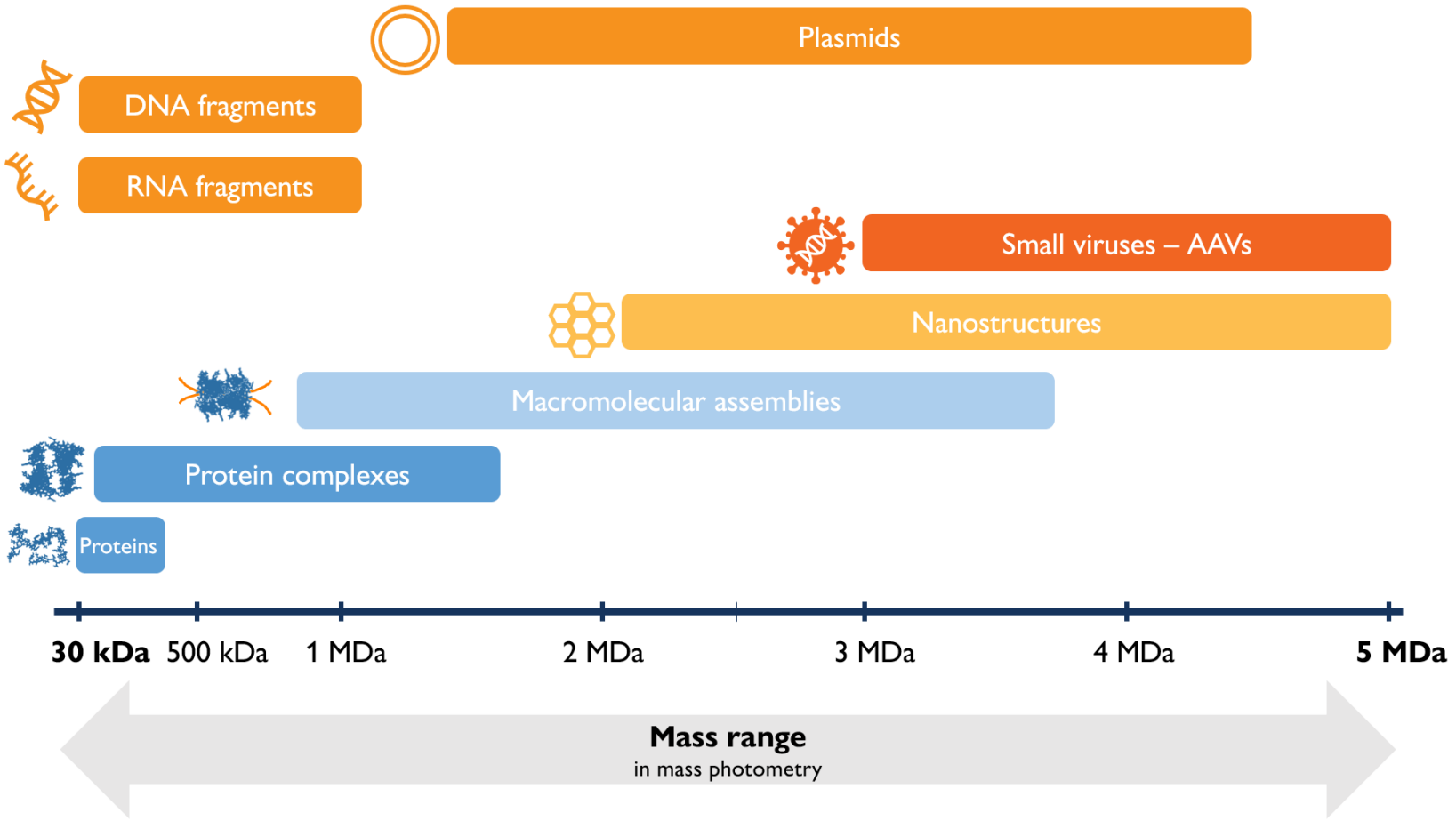




- **access to subpopulations** to determine complex stoichic and oligomeric state
- the monitoring of **complex, multistep processes**
- detection of **low abundance species**
- the characterization & monitoring of **sample heterogeneity**

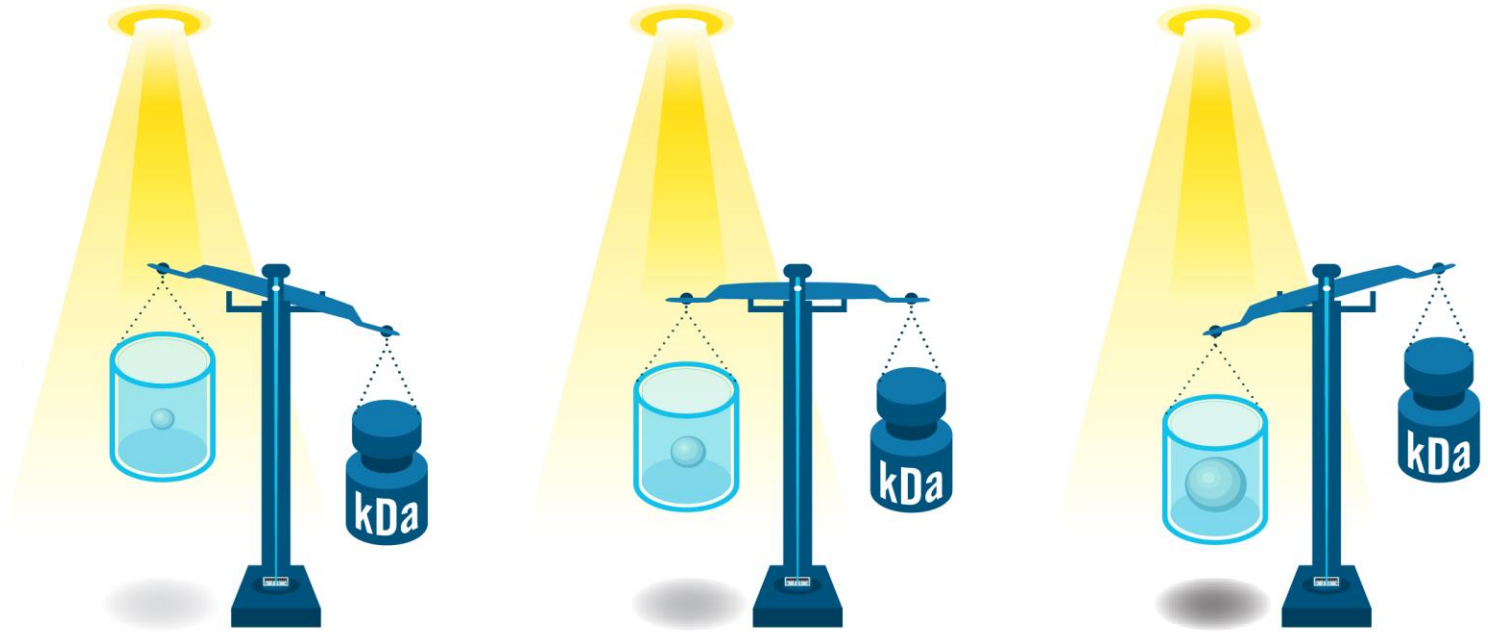
Data courtesy of Weston Struwe, Univ. of Oxford and Refeyn team

Mass photometry measures different types of biomolecules



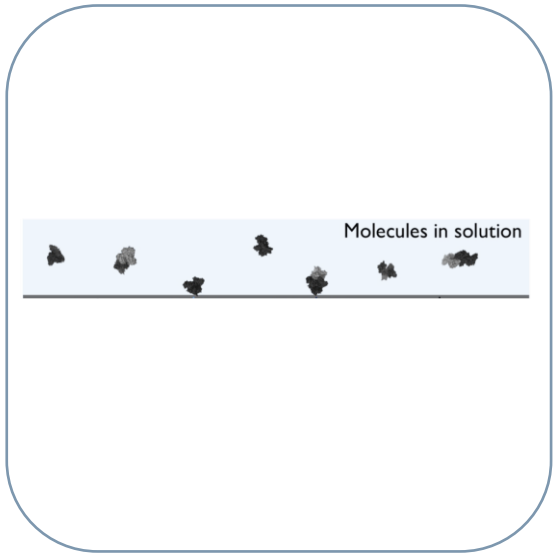
What does a mass photometry measurement look like?

Mass photometry weighs molecules with light



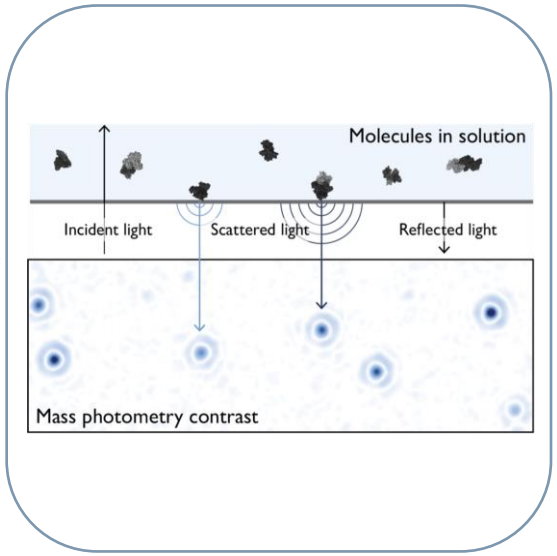
How does mass photometry work?

1



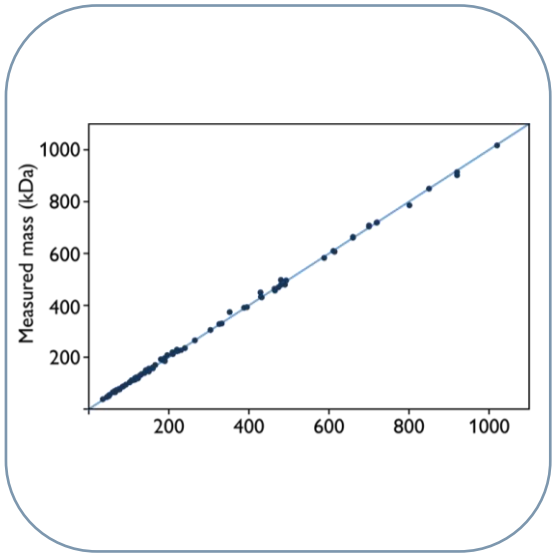
Biomolecules approaching a glass surface are illuminated by a laser

2



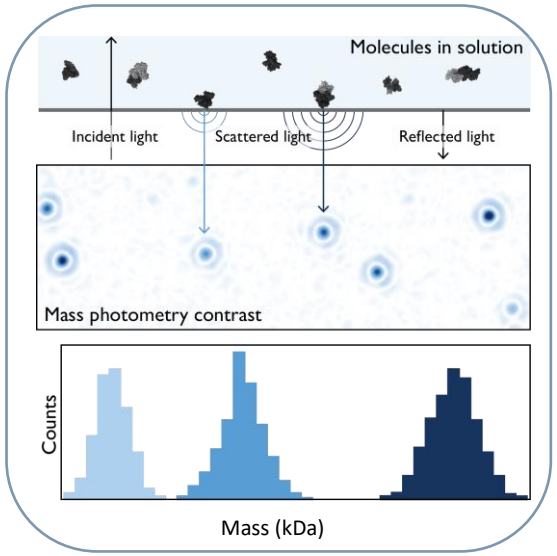
The biomolecules scatter light, which interferes with the light reflected at the interface

3



The resulting interface contrast scales linearly with the mass of the biomolecule

4



A mass histogram is generated from the single molecule measurements

Mass photometry in biophysical characterization

Meet the family!

Samux^{MP}

Optimized for AAV characterization

GMP Compliance coming soon!

Two^{MP}Auto

Automated mass characterization

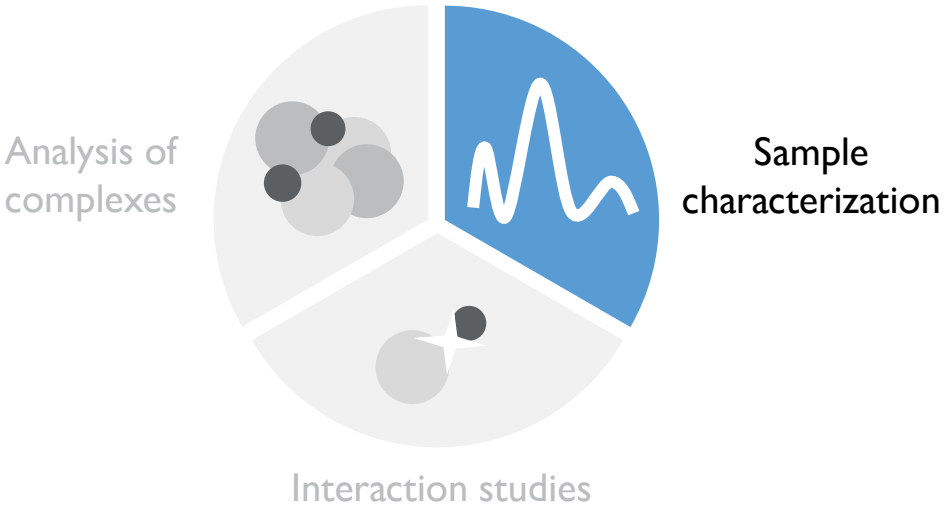
Two^{MP}

Second-generation mass photometer for single-molecule mass measurements

Samux^{MP}

A mass photometer optimized for AAV characterization

- Easily and accurately assess empty-full AAV ratios
- Quantify partially filled & overfilled particles
- GMP compliance coming soon

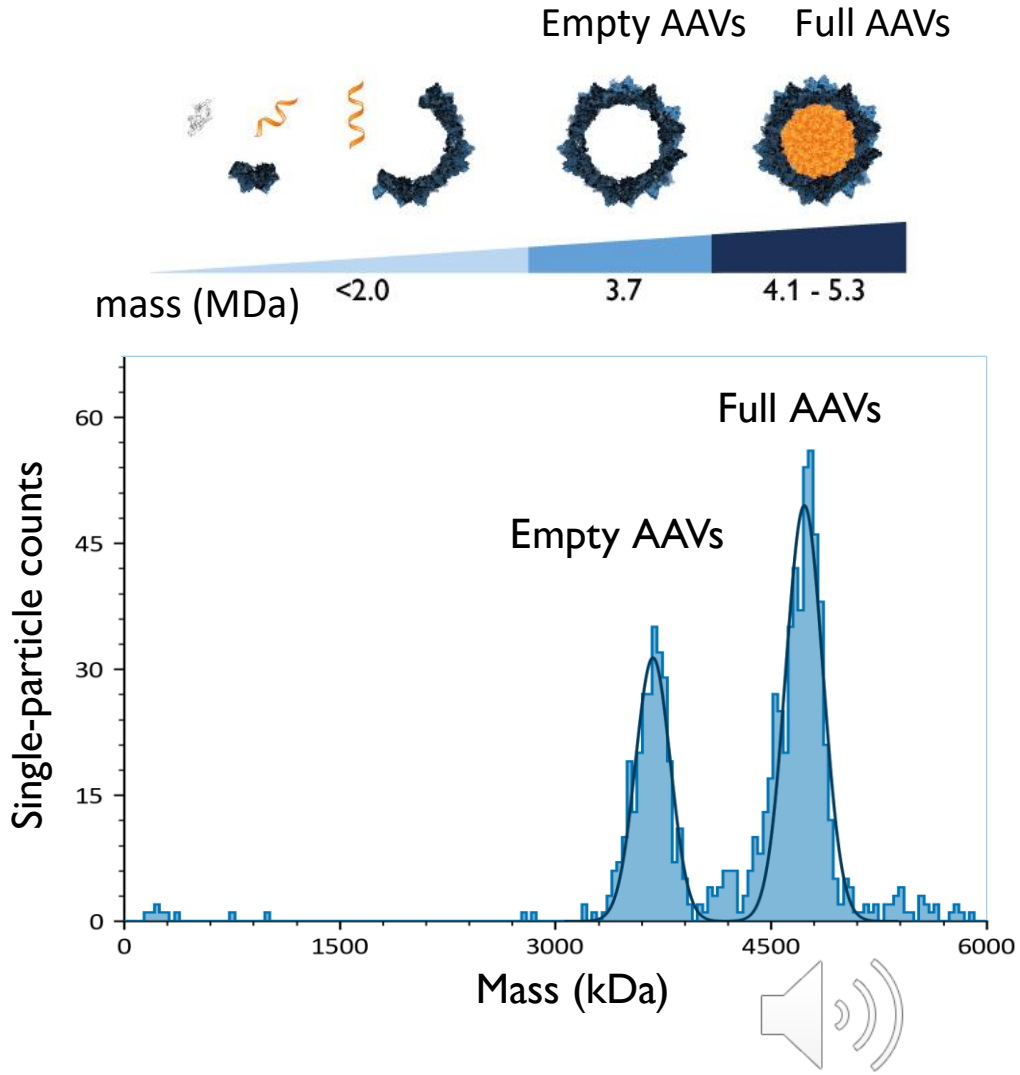


with rapid, single particle, serotype-agnostic
mass measurement of capsids in their native state, using
minimal sample consumption and with low operational costs

Samux^{MP} specifications

GMP Compliance coming soon!

Mass range	500 kDa – 6 MDa
Optimal concentration	10^{11} particles/mL
Sample volume	10-20 µL
Resolution (FWHM)	235 kDa @ 3700 kDa
Measurement time	< 5 minutes
Laser wavelength	488 nm



Samux^{MP} comparison to the gold standards cryo-TEM and AUC

	E : F calculation (% Full)		
Sample	Cryo-TEM	AUC	Samux^{MP}
1	6 %	5 %	5 %
2	18 %	28 %	18 %
3	24 %	35 %	30 %
4	60 %	-	53 %

	E : F calculation (% Empty)		
Sample	Cryo-TEM	AUC	Samux^{MP}
A	16 %	24 %	16 %
B	-	100 %	95 %

Performed at
Pharmaron Gene Therapy,
Liverpool, UK

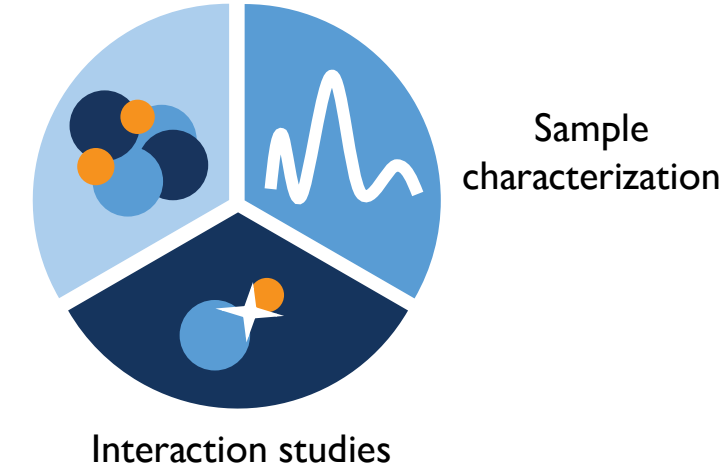
Benchmarking

- E:F ratios of multiple AAV preps of different serotypes were measured with:
  - electron microscopy (cryo-TEM)
  - analytical ultracentrifugation (AUC)
  - Samux^{MP} mass photometry
- The results from Samux^{MP} agreed with the results from AUC and cryo-TEM

Two^{MP}

A versatile mass photometer with a wide range of biophysical applications

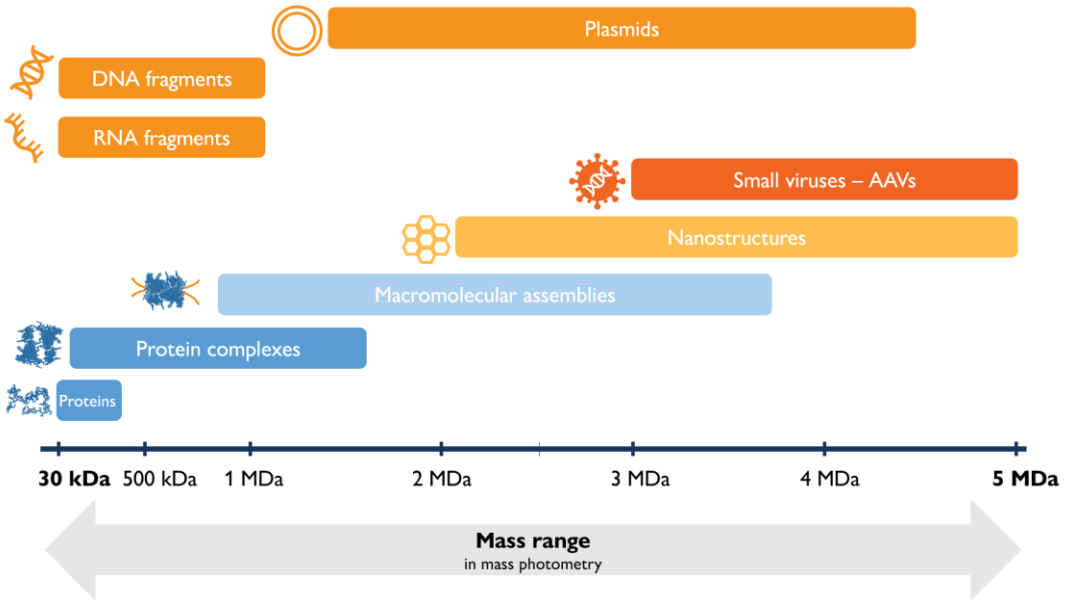
Detect **low abundance species** and verify **sample purity**
Characterise **sample heterogeneity** and determine **filled ratios**
Determine **oligomeric states** & characterise **complexes**
Monitor **complex, multistep processes** & quantify **biomolecule interactions**



with a rapid, single-molecule mass measurement of label-free biomolecules in their native state, over a wide mass range with minimal sample consumption

Two^{MP}

A versatile mass photometer with a wide range of biophysical applications



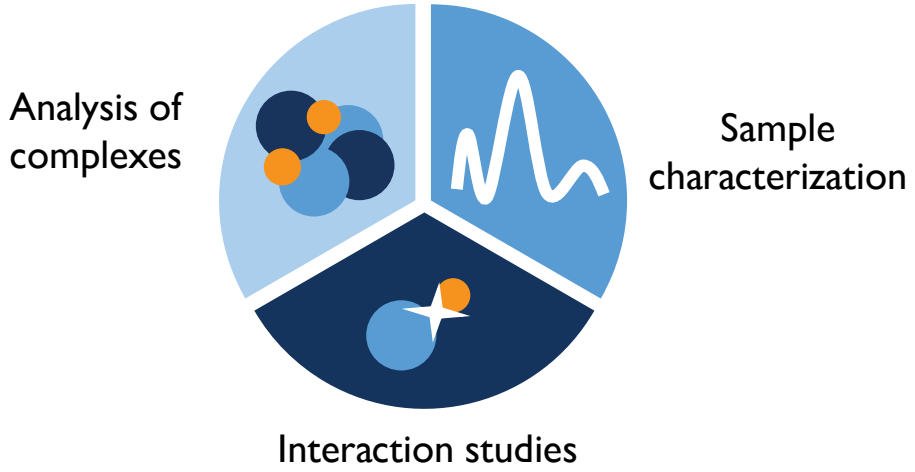
Mass range	30 kDa – 5 MDa
Concentration range	100 pM – 100 nM
Sensitivity	<< 1 ng of protein
Resolution (FWHM)	25 kDa @ 66 kDa
	60 kDa @ 660 kDa
Mass precision	± 2%
Mass error	± 5% (single measurement)

Two^{MP} Auto

Automated mass characterization of biomolecules

- Greater data confidence
- Reduced operator time
- Ideal for screening and titration assays

+ standard mass photometry applications

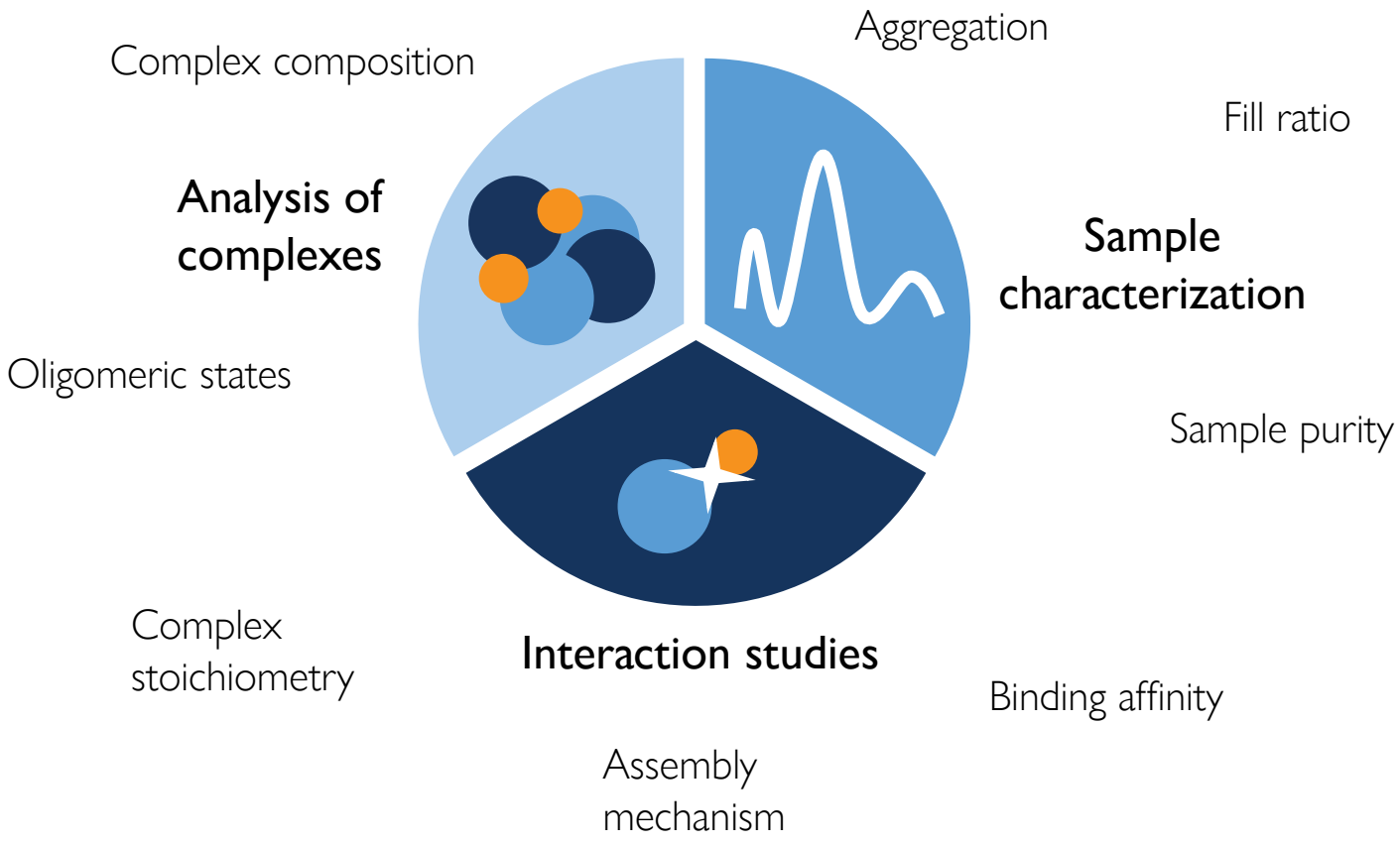


with rapid, autonomous measurement of multiple samples and increased data reproducibility

- Up to 1 hour of autonomous measurement possible
- Compatible with Two^{MP} systems
- Same specifications as Two^{MP}*

**or One^{MP} if an upgraded system*

Two^{MP} and Two^{MP} Auto applications



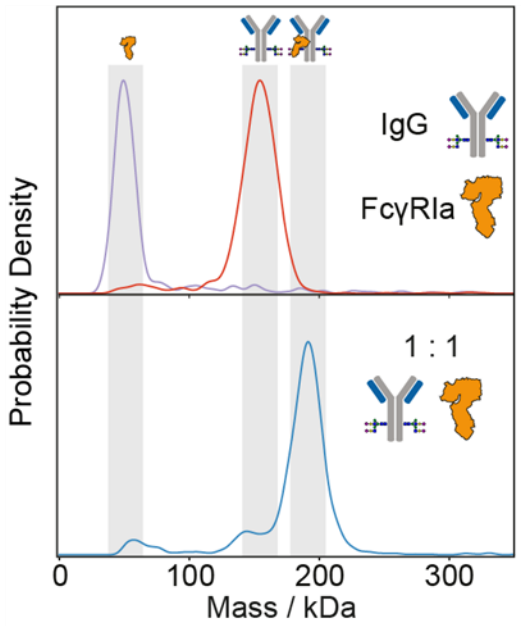
Mass photometry can quantify protein interactions

What is the binding affinity?

Determining the binding affinity of an antibody with another protein in a single-shot K_d

1:1 mixture of IgG & FcγRIa

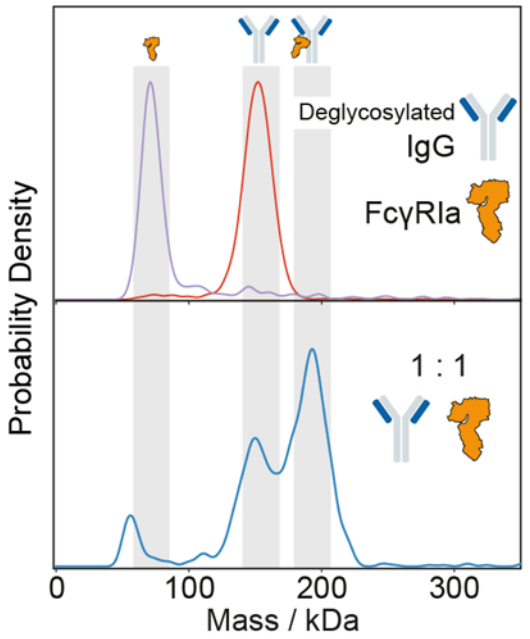
→ dissociation constant information



K_d = 0.05 ± 0.01 nM

1:1 mixture of deglycosylated IgG (Fc N-glycan removal) & FcγRIa

→ 20-fold change in binding affinity



$K_d = \frac{[\ ][\ ]}{[\ ]}$

K_d = 1.0 ± 0.1 nM

Measured on the One^{MP}

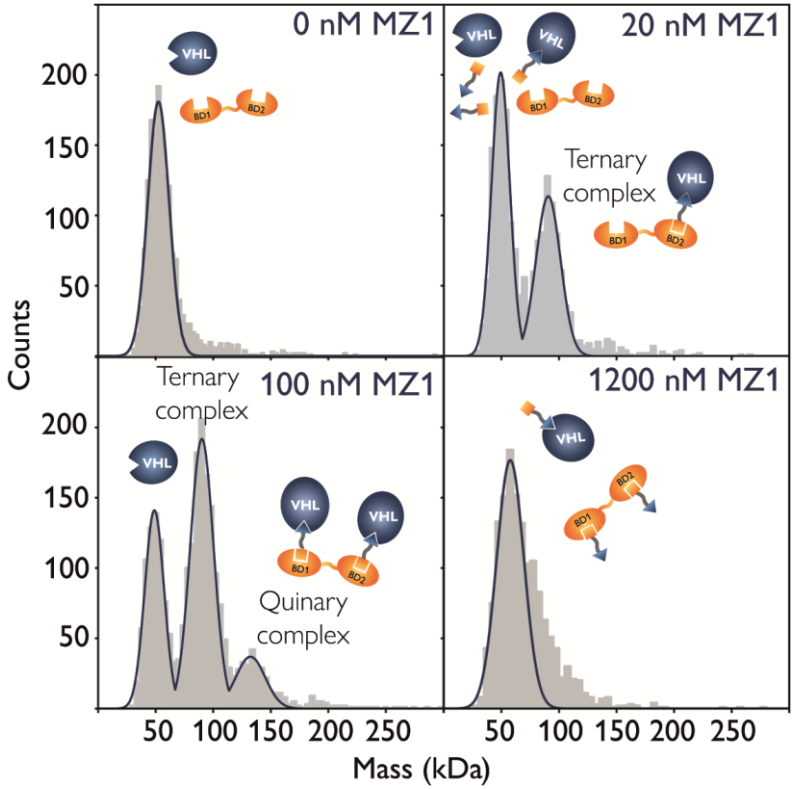
Soltermann et al., Angew Chem Int Ed Engl. 2020

Mass photometry enables PROTAC characterization

How does the ternary complex form?

- Characterization of small bifunctional molecule (PROTAC) binding
- Ternary complex formation measured as a function of MZ1 (PROTAC) concentration

1. Target protein (with binding sites BD1 & BD2) + E3 ligase (VHL)



2. PROTAC binds E3 ligase to binding site on target protein

→ ternary complex forms

3. PROTAC **binds** E3 ligases to both binding sites on target protein

→ quinary complex forms

4. Saturation of binding sites at target protein & ligase

→ inhibition of complex formation at 1200 nM MZ1

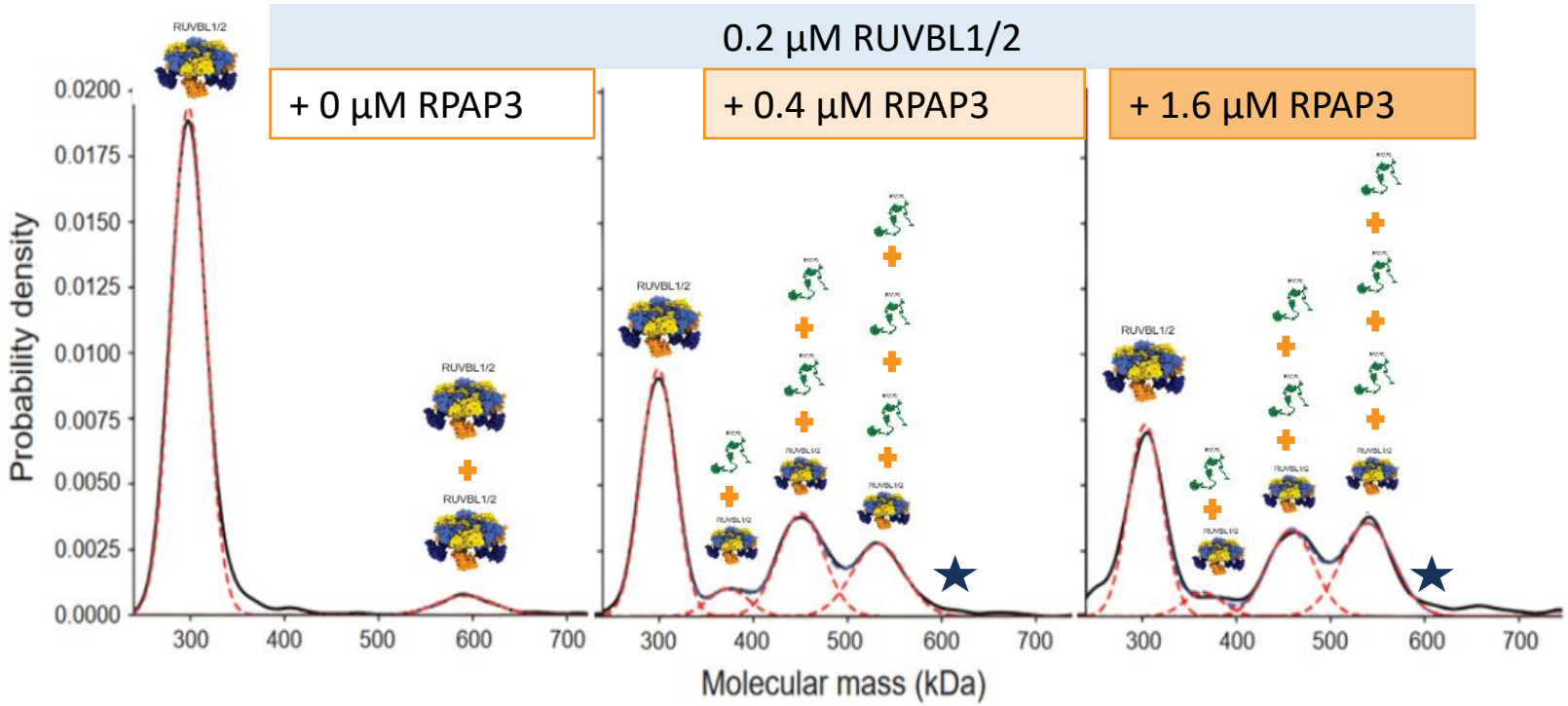
Measured on the One^{MP}

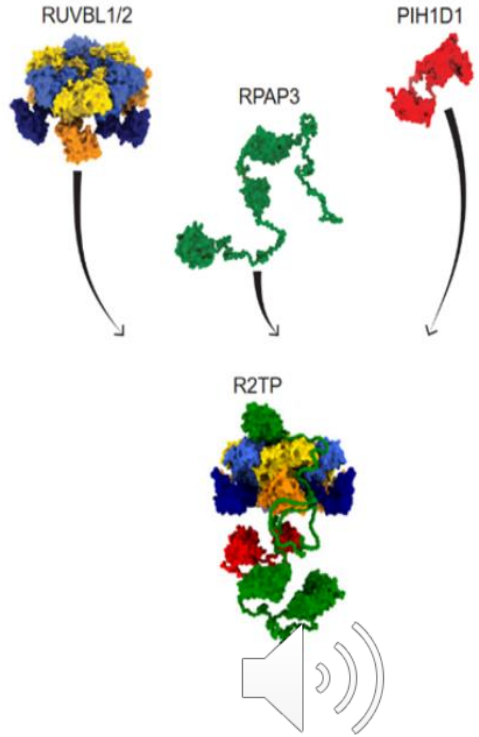
[Refeyn Application Note - PROTACs]

Mass photometry tracks the assembly of complexes

How do the binding partners influence oligomeric assembly?

- Mass photometry was used to determine the subunit stoichiometry within the R2TP chaperone complex
- Stoichiometry of oligomeric assembly revealed by titration of binding partners





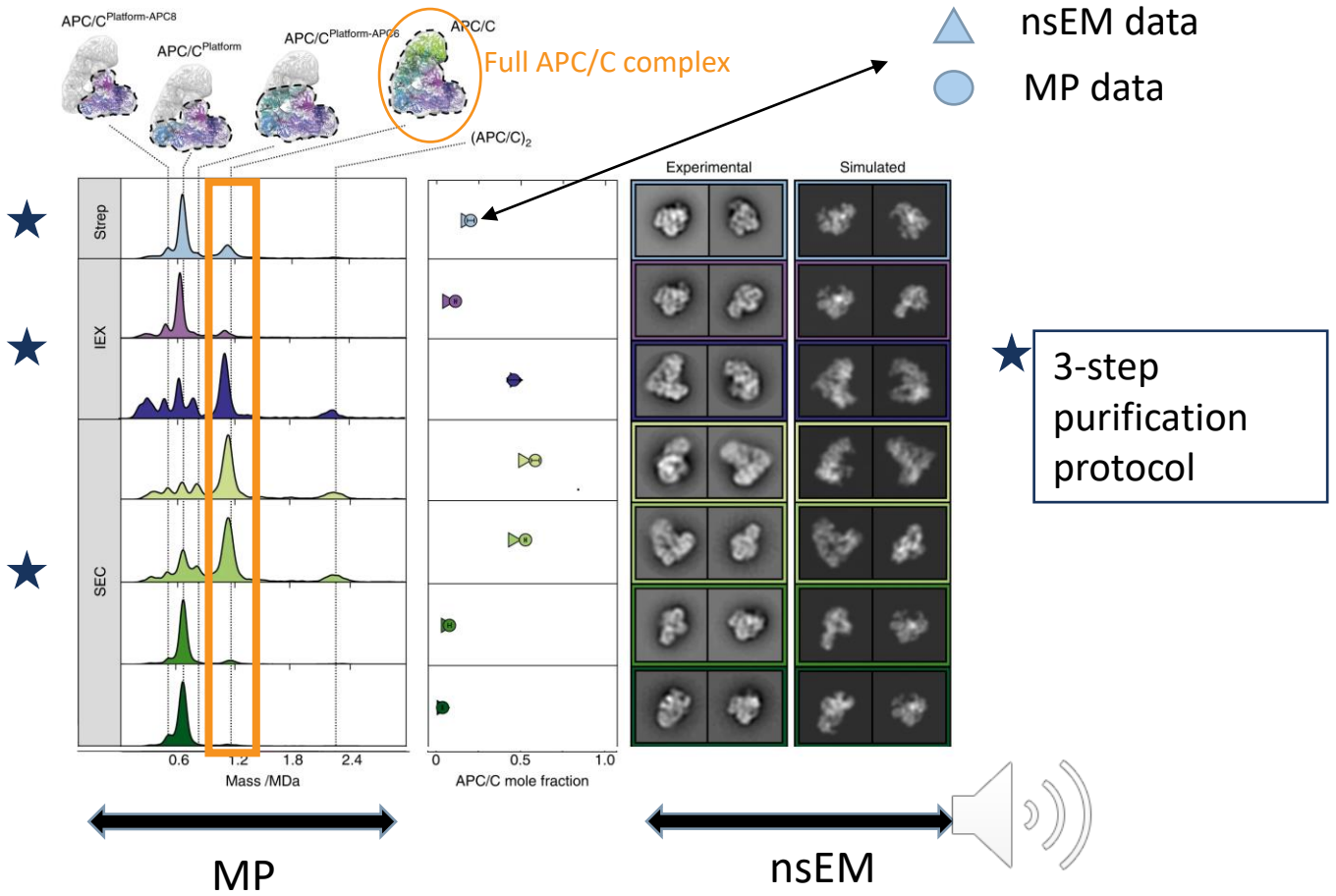
Measured on the One^{MP}

Seraphim et al., Structure, 2022

Mass photometry can quantify sample heterogeneity

Which chromatography fractions contain most of the fully assembled complex?

- Purification of the Anaphase Promoting Complex / Cyclosome (APC/C) is a multistep process
- Mass photometry provided clear information on native assembly quickly & using very little sample (15 µL of typically 5-25 nM protein)
- Perfect agreement with nsEM – but easier + faster



Measured on the One^MP

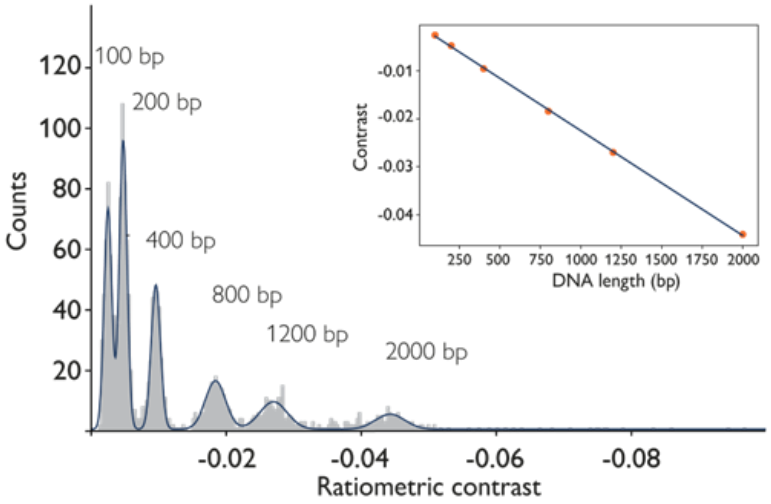
Sonn-Segev et.al, Nat Comm 2020

Mass photometry measures nucleic acids

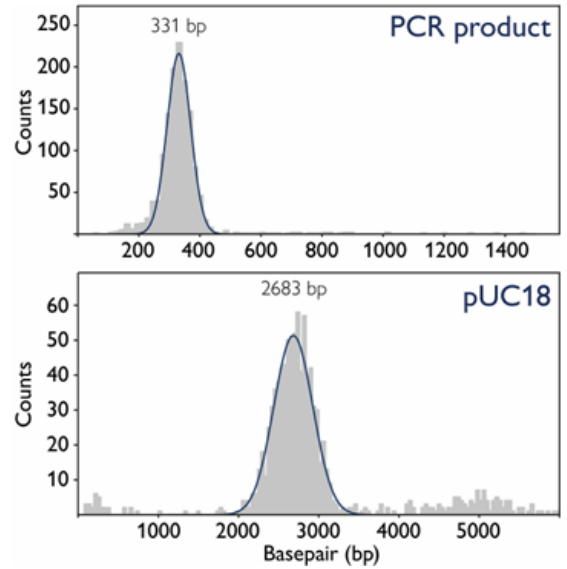
Nucleic acid sizing

- Mass photometry can be applied to nucleic acids and other biomolecules
- Same linear dependence between measured contrast & length/mass
- Here, the coverslip was coated in PLL to enable DNA binding

DNA calibration



Unknown DNA lengths determined



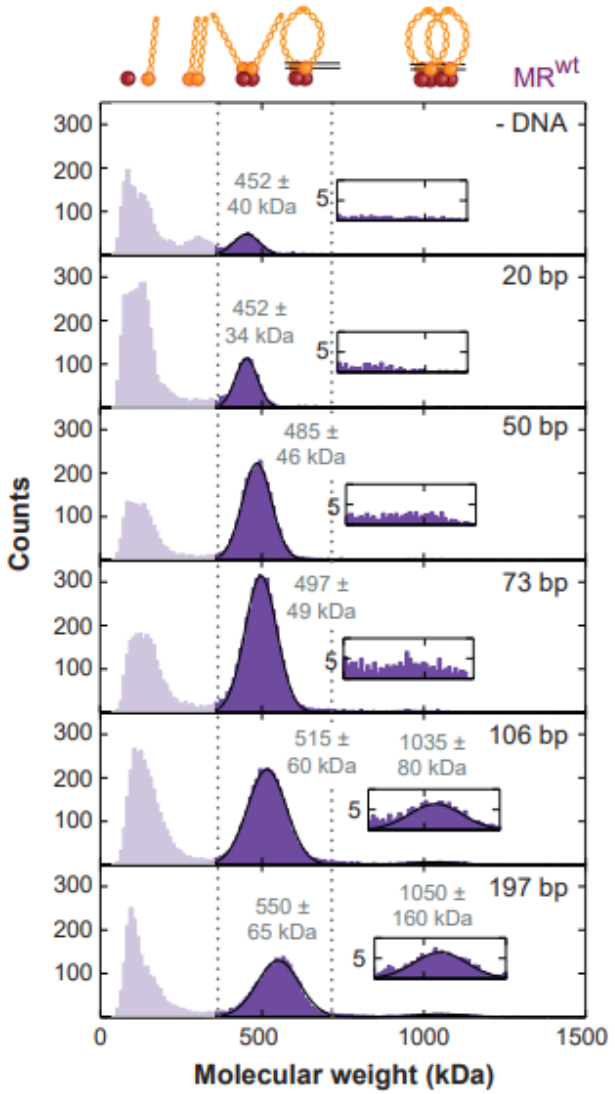
Measured on the One^{MP}

Application Note – Mass Photometry of Nucleic Acids

Mass photometry reveals protein-DNA interactions

What is the MR-DNA assembly mechanism in double-strand repair?

- Determine how many proteins bind to a DNA sequence
- Small changes in protein/DNA ratio clearly observable
- It is straightforward to assess how the DNA-protein complex forms
- It is possible to study MR complex in double-strand repair



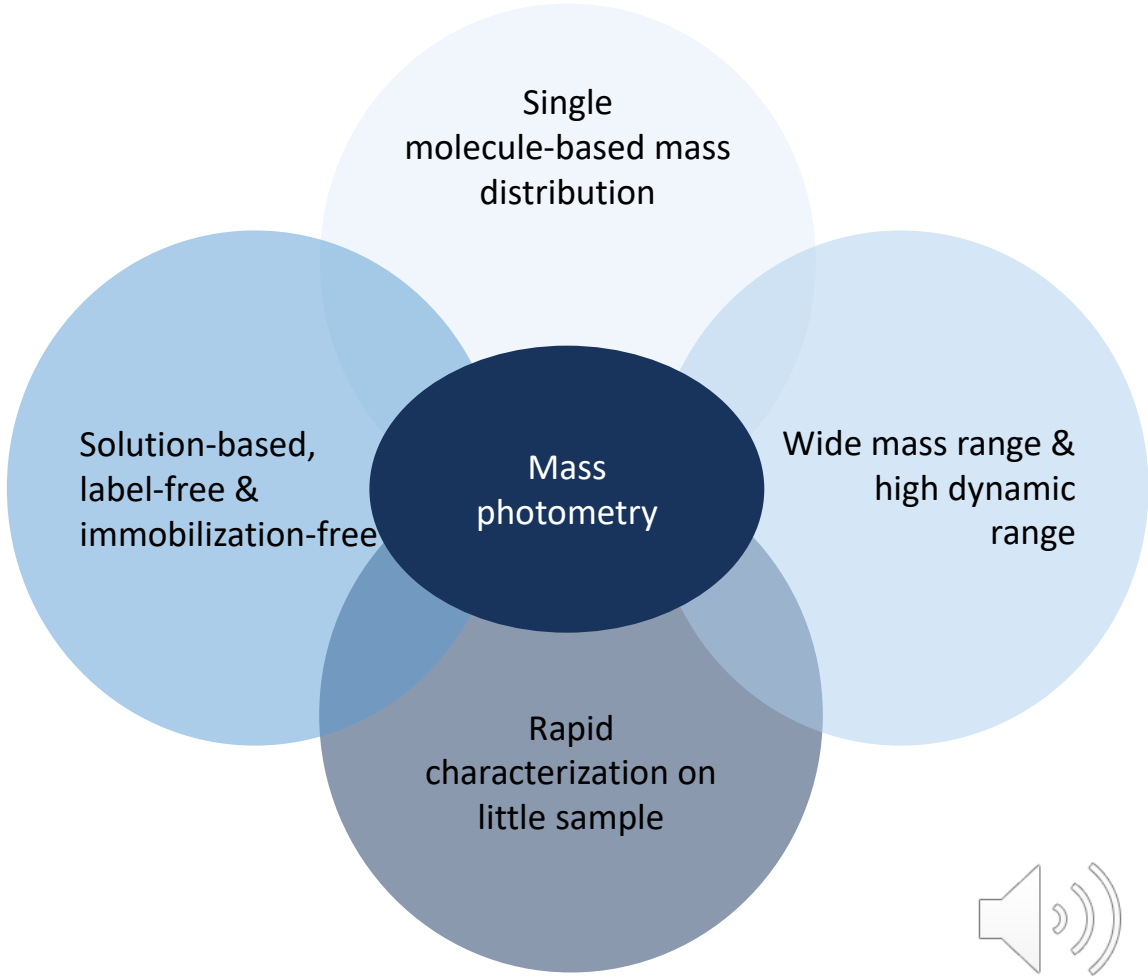
Measured on the One^MP

Kissling, et al., Nat Comm 2022

Mass photometry in biophysical characterization

Single-molecule counting over a wide mass range & high dynamic range of **label-free** biomolecules in their native state

- Applicable to many biological questions
- Quick & easy to use
- Cost effective



Want to learn more about mass photometry?

Come see us at Booth 26!

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