Experimental Phasing:

Rational Approaches to Heavy Atom Derivatives of Proteins

Mathias Gruen, Christin Reuter, Larissa Consani Textor, Thomas Billert

Jena Bioscience GmbH
Löbstedter Str. 80
07749 Jena, Germany
Tel.: +49-3641-628-5000
Fax: +49-3641-628-5100
e-Mail: info@jenabioscience.com

Experimental phasing still important for protein crystallographers

37 % of all known protein structures, and 21 % of all new structures since 2011 solved by experimental phasing (source: pdb data base)

Ideally: Molecular Replacement (MR)

- fast & easy
- but not successful in 20…40% of all cases

Second line of defense: Experimental Phasing

- until the 1990s: MIR* and SIRAS*
- from 1990 : MAD*
- from 2000: SAD*

*MIR = Multiple isomorphous replacement
MAD = Multiple wavelength anomalous dispersion
SIRAS = Single isomorphous replacement plus anomalous scattering
SAD = Single wavelength anomalous dispersion

Experimental phasing does require derivatization of protein (crystals) with heavy atoms

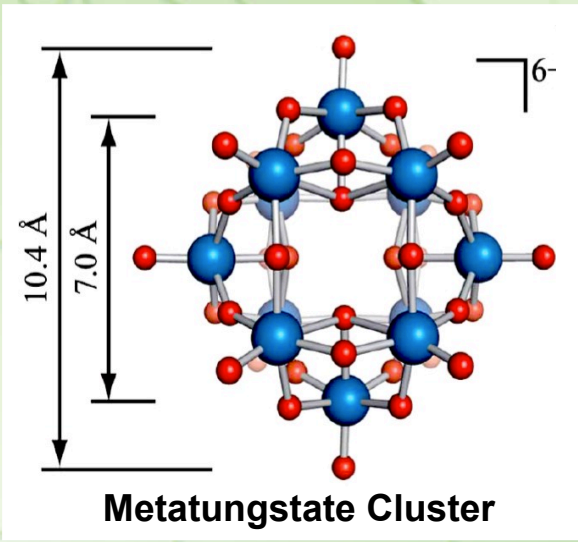
3 modes of heavy atom protein derivatization to talk about today

Tantalum and Tungsten Cluster Derivatization

JBS Magic Triangle

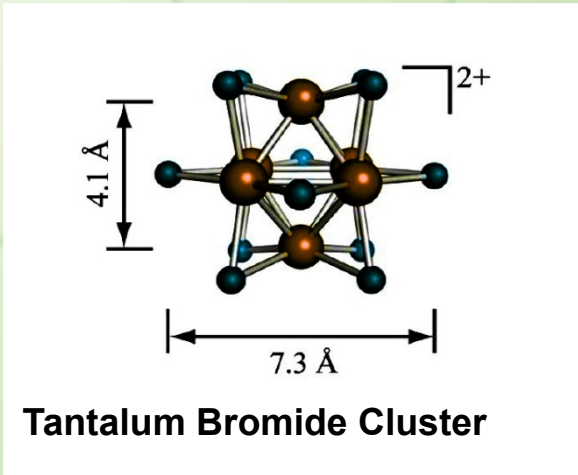
Heavy atom containing Nucleotides and Oligos

„Scattering super heavy atoms“: Tungstate and TaBr-Clusters



Metatungstate Cluster

- 12 tungsten atoms bridged by oxygens:
  - “super heavy atom”
  - W anomalous scattering for SAD & MAD
- Highly soluble in water
- Available in 3 types with different final charges for optimal binding to positively charged patches of the protein
  - Phosphotungstate (charge 3-)
  - Metatungstate (charge 6-)
  - Paratungstate (charge 10-)



Tantalum Bromide Cluster

- 6 tantalum atoms clustered by 12 bromines:
  - “super heavy atom”
  - Ta and Br anomalous scattering for SAD & MAD
- Soluble in aqueous solutions and stable over a wide pH range

Suitable for in-house X-ray generator, increase chances of phasing of low resolution data sets

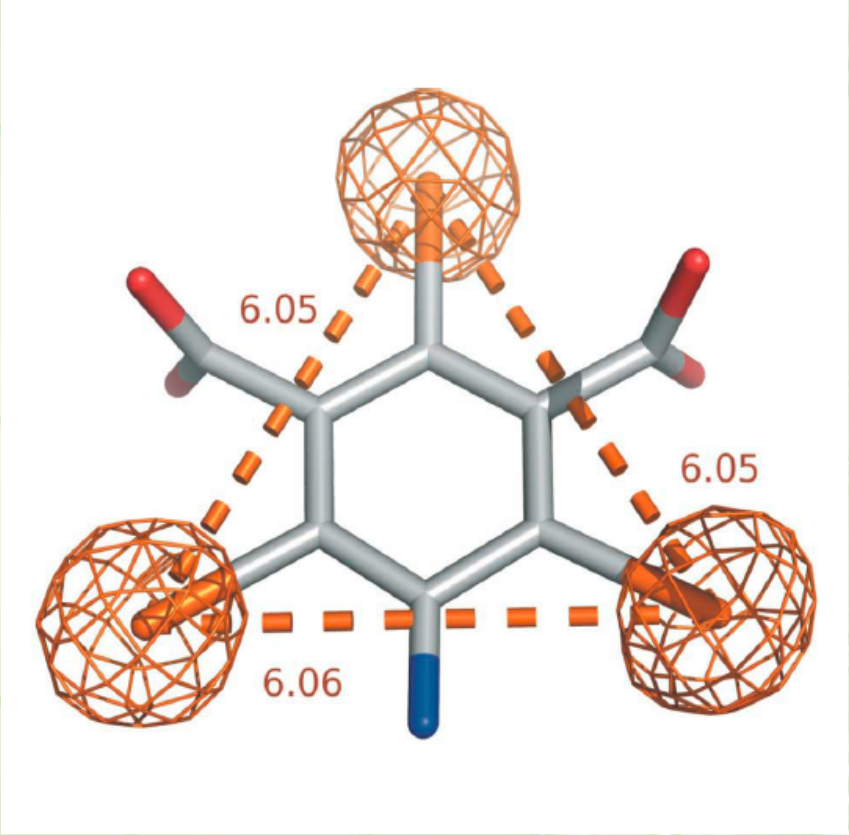
3 modes of heavy atom protein derivatization to talk about today

Tantalum and Tungsten Cluster Derivatization

JBS Magic Triangle

Heavy atom containing Nucleotides and Oligos

5-Amino-2,4,6-triiodoisophthalic acid is termed the "Magic" Triangle



- Three iodine atoms useful for SAD and SIRAS
- High occupancy in protein crystal
  - carboxylates ➔ interact with positive side chains
  - amino group ➔ interacts with negative side chains
  - Iodines support crystal growth via H-bonds
- Equilateral triangle easily identifiable during substructure determination

Again no travelling to synchrotron: Suitable for in-house X-ray generators

3 modes of heavy atom protein derivatization to talk about today

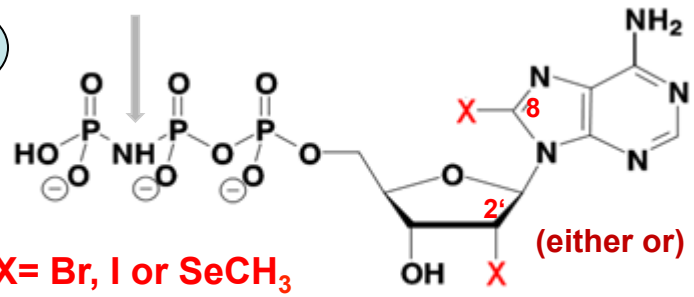
Tantalum and Tungsten Cluster Derivatization

JBS Magic Triangle

Heavy atom containing Nucleotides and Oligos

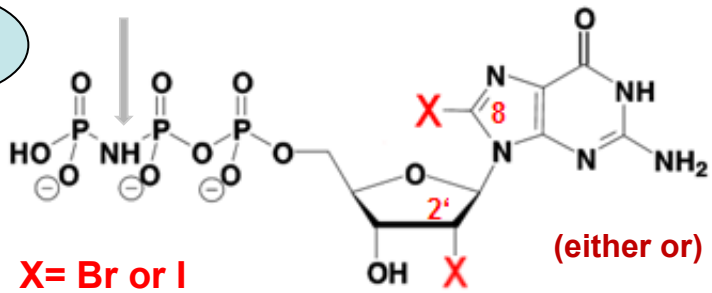
Hg, Se, I and Br easily incorporated into nucleoside triphosphate binding enzymes

ATP



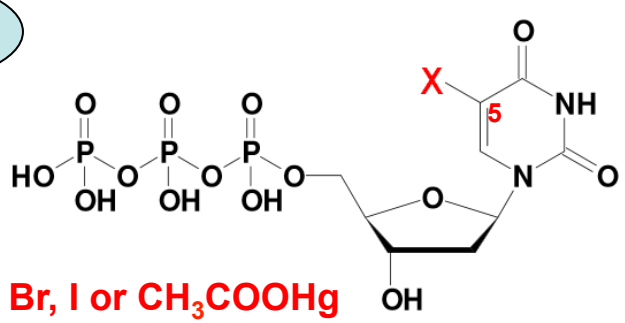
X= Br, I or $SeCH_3$

GTP



X= Br or I

U/CTP



X= Br, I or CH_3COOHg

For ATP-binding enzymes such as kinases, motor proteins, chaperones, …

- 2'-substitutes used for solving structure of thymidine kinase
- 8-substitution is tolerated by heat shock proteins (DnaK)

For GTP-binding signal transduction enzymes such as small GTPases, heterotrimeric, …

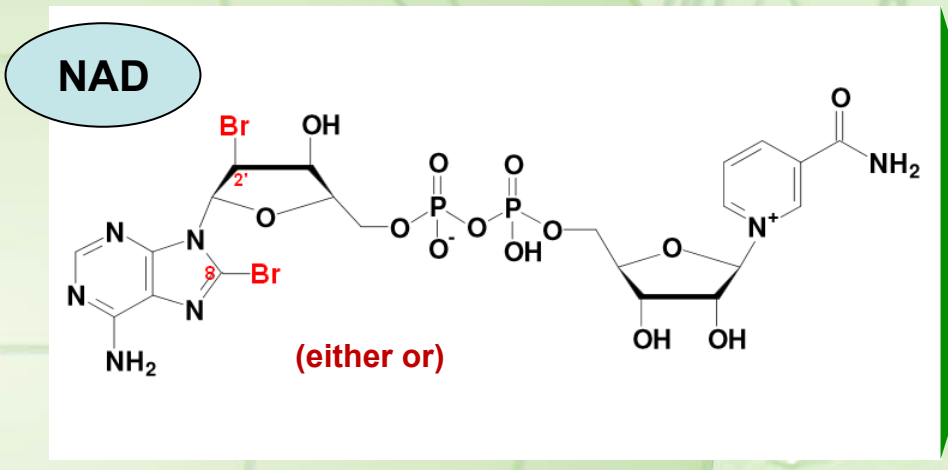
- 2'-substitutes used for p21ras and Rab5

For DNA polymerases and nucleotidyl transferases

- used for thymidylate synthase-complementing proteins (TSCPs)

Method is limited to NTP-binding enzymes, but many "important" enzymes do bind NTPs

Brominated NAD(1) mimics the natural coenzyme NAD(1)



- For enzymes involved in cellular redox processes such as glycolysis, nucleic acid and lipid biosynthesis
- Already used as substrate for ADP ribosyl cyclase

Similar approach to modified NTPs but for NAD-binding enzymes

(1) NAD = Nicotinamidadeninedinucleotide

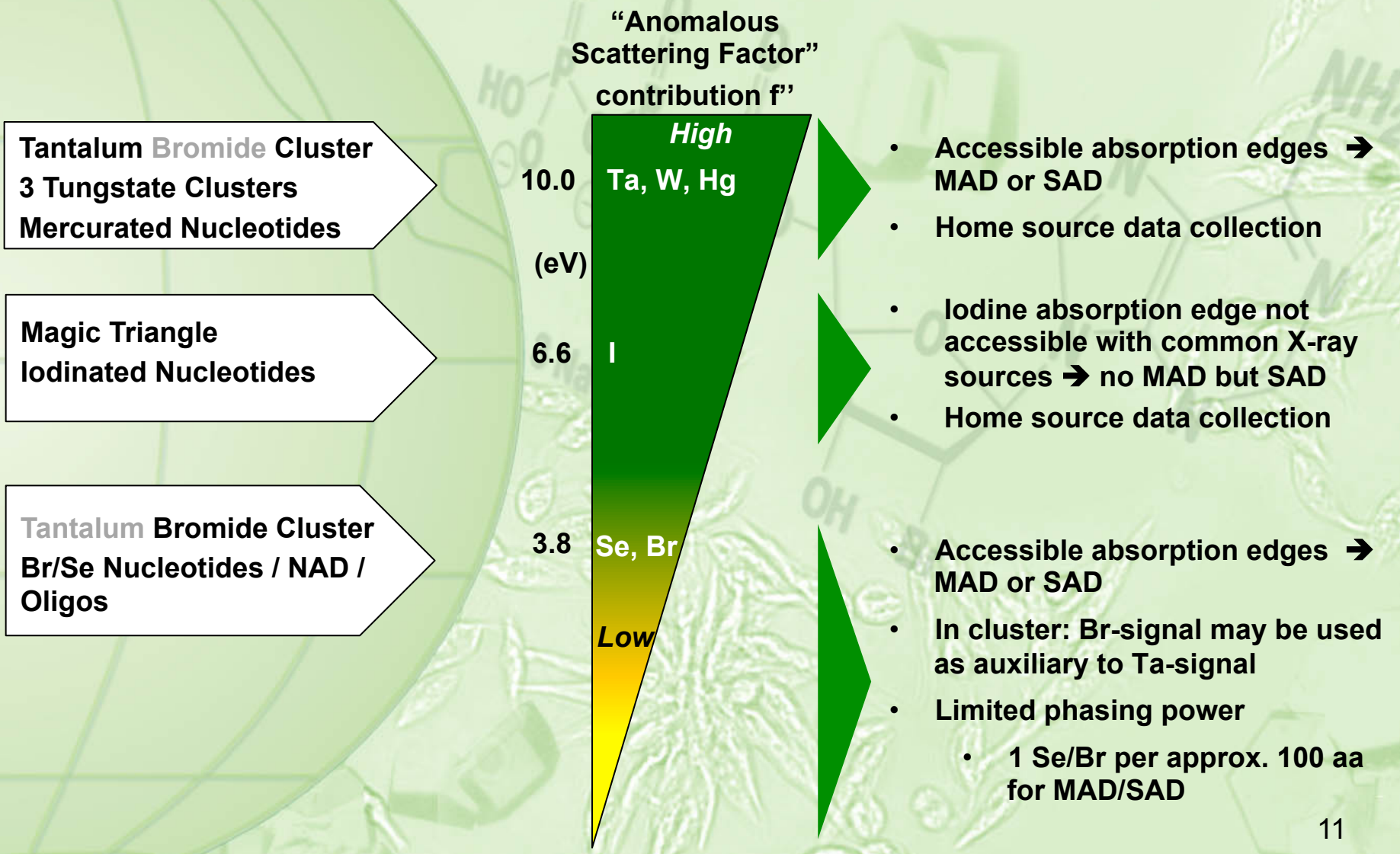
Brominated and iodinated oligos for phasing of protein-DNA complexes

Many DNA-binding proteins e.g. transcription factors or methyl transferase require co-crystallization with DNA anyway

Modification	Standard Purification				HPLC Purification			
	0.02 µmol 3 OD_{260}	0.04 µmol 5 OD_{260}	0.2 µmol 12 OD_{260}	1.0 µmol 50 OD_{260}	0.02 µmol 1 OD_{260}	0.04 µmol 2 OD_{260}	0.2 µmol 5 OD_{260}	1.0 µmol 12 OD_{260}
C8-Alkyne-dC	25 €	28 €	33 €	60 €	26 €	29 €	37 €	65 €
C8-Alkyne-dU	15 €	17 €	27 €	57 €	16 €	18 €	30 €	62 €
C2-Amino-dT	--	--	--	--	70 €	80 €	120 €	290 €
Biotin-dT *	--	--	--	--	95 €	105 €	145 €	360 €
Bromo-dC	--	--	--	--	78 €	91 €	104 €	208 €
Bromo-dG	--	--	--	--	116 €	116 €	149 €	238 €
Bromo-dU	--	--	--	--	78 €	91 €	104 €	208 €
7-Deaza-dA	--	--	--	--	76 €	76 €	105 €	228 €
2-Deoxyuridine	7.50 €	10 €	12 €	15 €	12.50 €	15 €	17.50 €	20 €
Inosine	7.50 €	10 €	12 €	15 €	12.50 €	15 €	17.50 €	20 €
Iodo-dC	--	--	--	--	116 €	116 €	210 €	332 €
Iodo-dU	--	--	--	--	83 €	83 €	105 €	166 €
Methylcytosin	15 €	17 €	20 €	40 €	17.50 €	20 €	25 €	50 €

Py-5 substitutions do not disrupt Watson-Crick base pairing; G-8 substitution induces *syn* pairing geometry often used in structural studies of DNA conformation

Take home message: There are easy-to-use tools available avoiding tedious (and toxic…) trial-and-error HA-screening…



Acknowledgements

Manuel Than Sven Dahms Carsten Streb

Tobias Beck

proteros

Jena Bioscience
www.jenabioscience.com

Christin Reuter

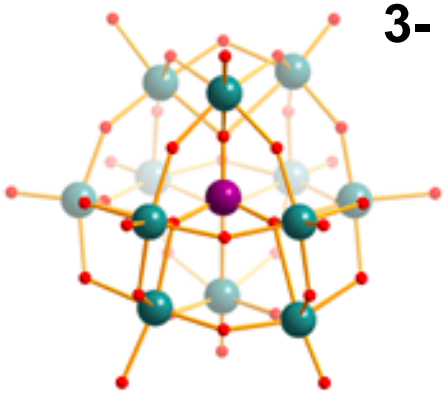
Larissa Consani
Textor

Thomas Billert

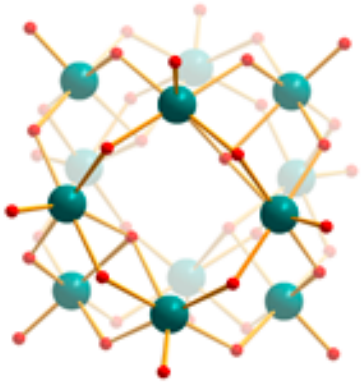
unused

Protein binding of Tungstate cluster may be optimized by variation of charge and shape

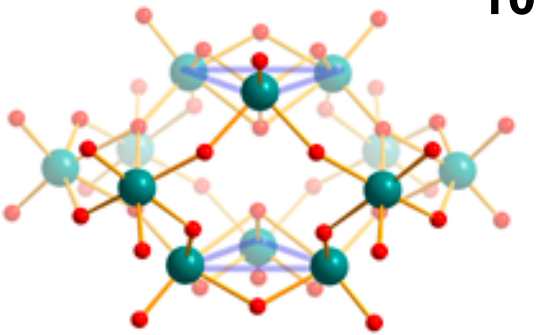
Phosphotungstate 3-



Metatungstate 6-



Paratungstate 10-



The Tungstate Cluster Kits consist of 6 ready-to-use aliquots of Phosphotungstate, Metatungstate or Paratungstate salts, respectively. All Tungsten clusters contain 12 Tungsten metal centers bridged by Oxygen atoms, but differ in their resulting negative charge (3-, 6- and 10-, respectively).

Guideline for designing experimental phasing with HA clusters

Solubility of HA clusters depends on the precipitant solution	Screen for optimal soaking conditions and/or use a different HA cluster
Cluster degradation during crystal derivatization may lead to cluster sites with low occupancy	Screen for soaking conditions in which the HA cluster is more stable and/or use a different HA cluster
Non-isomorphism due to crystal derivatization	Collect diffraction data using single or multi-wavelength anomalous diffraction approaches
Diffraction data quality insufficient to identify single cluster atoms	Use Tantalum Bromide cluster and take advantage of the Ta and Br anomalous signal

Anomalous scattering increases with atomic number Z