Encoding in Chemistry and Biology

1 2 3 4 5

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Nano Science Center, University of Copenhagen

Encoding in chemistry and biology

Biological encoding (DNA)
Chemical encoding
Encoding by position
Optical pattern encoding (barcodes)
Color encoding
Rf - encoding
Magnetic encoding
Spectroscopic encoding

Ease of preparation
Ease of handling
Cost
Feasibility
Chemistry compatibility
Speed of decoding
Assay compatibility
Reliability

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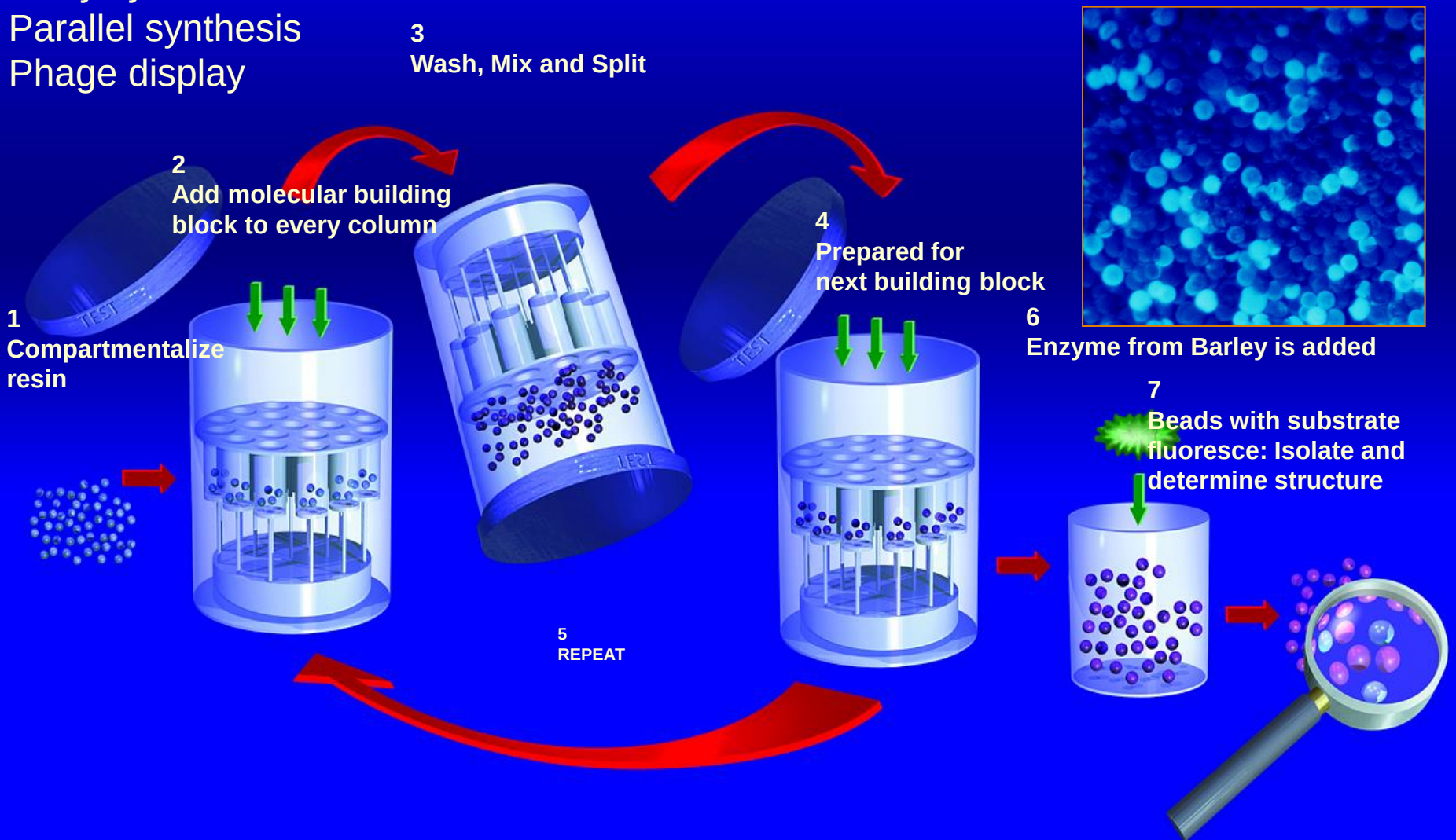
Split mix

Array synthesis

Parallel synthesis

Phage display



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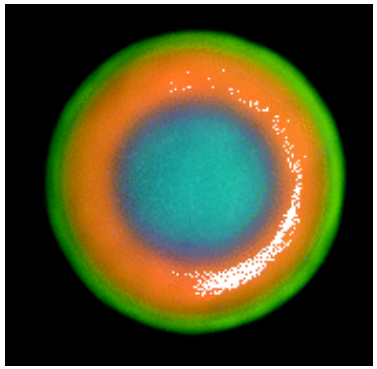
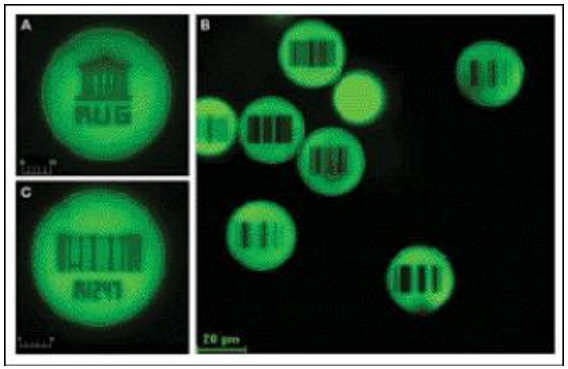
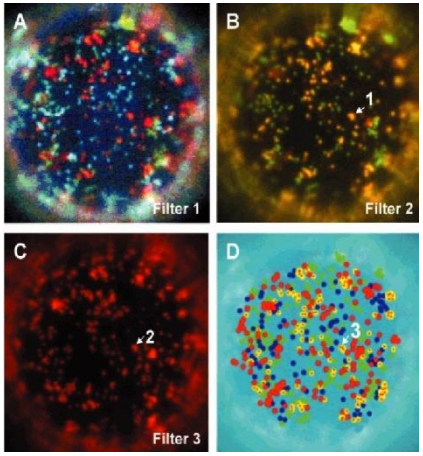


SPHERECODES



BARCODES (Tibotec, Ghent Uni.)

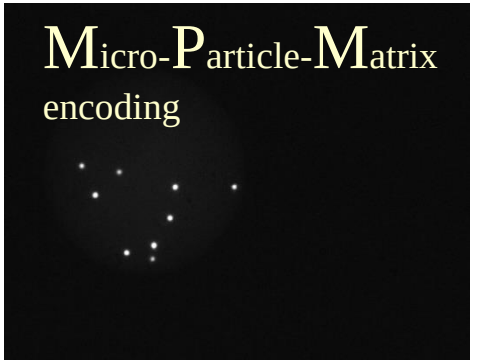


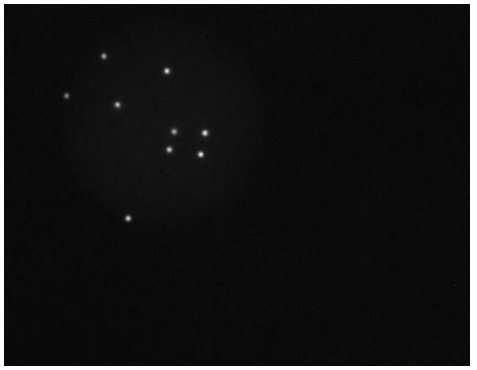
NANOPARTICLES

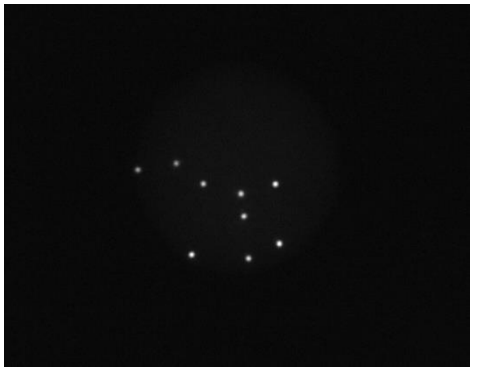
- Spherical encoding by diffusion, Miller et al.
- Laser etched barcodes, Leblans et al.
- Nanoparticle in situ encoding, Battersby et al.
- Infra red tags
- Binary fluorescence encoding

Discovery Partners Int.*
Encore International Corp.
Luminex*
Quantum dot*
Smart Bead Technologies Ltd.*
Spectra science corporation
Vitra Bioscience
Illumina
and many more ...

Micro-Particle-Matrix encoding

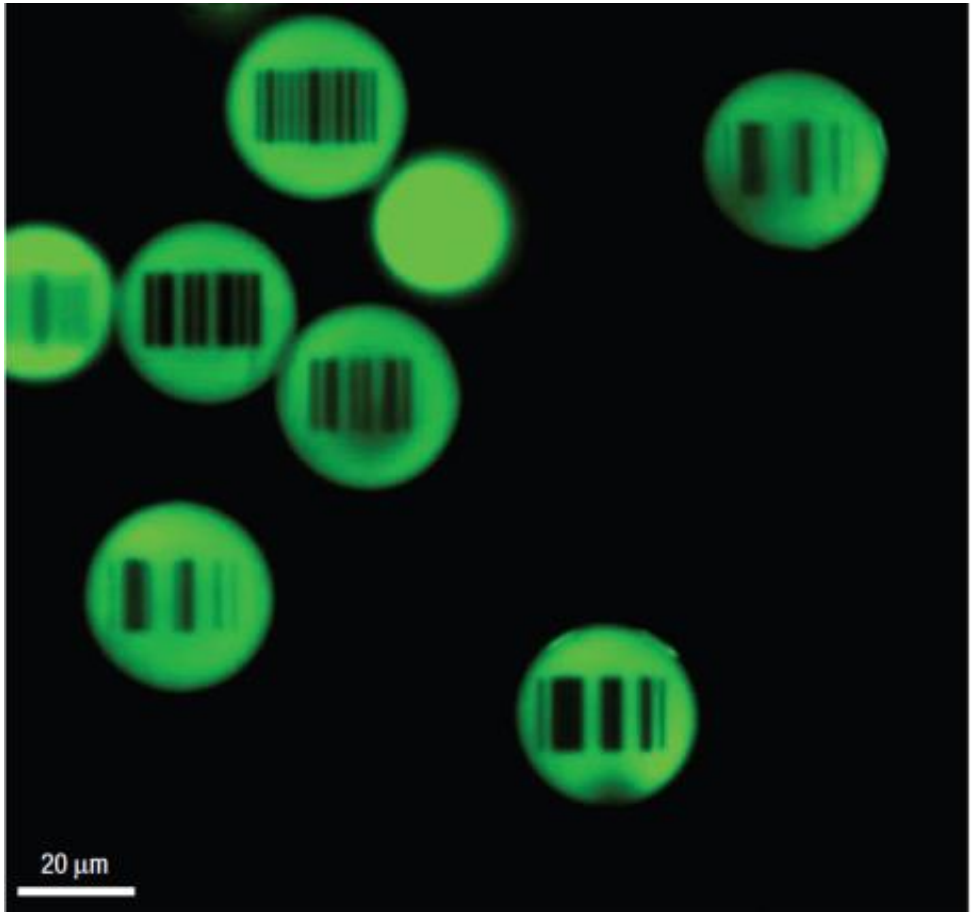


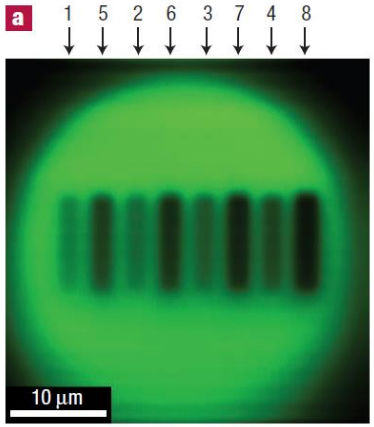


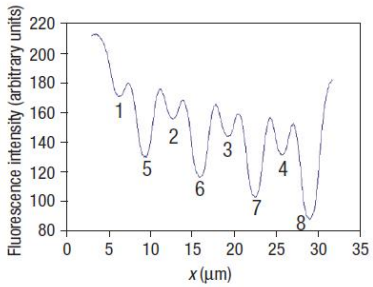


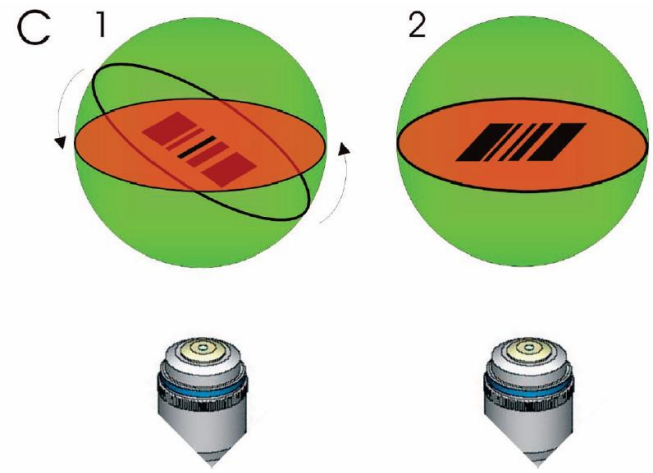
BARCODES (Tibotec, Ghent Uni.)

Code reads by confocal microscopy









Code reads problematic
Time consuming

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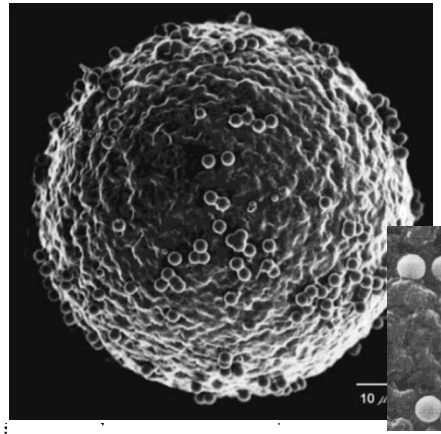
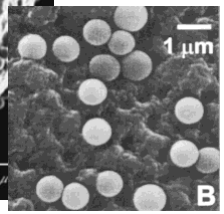
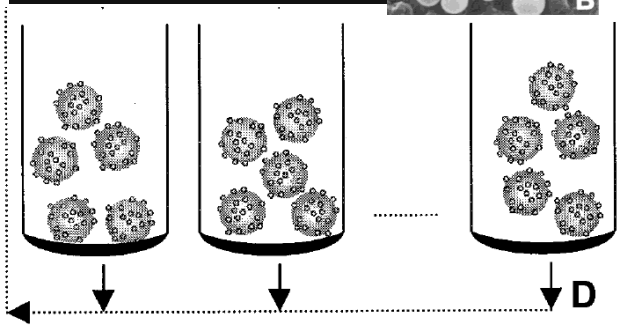






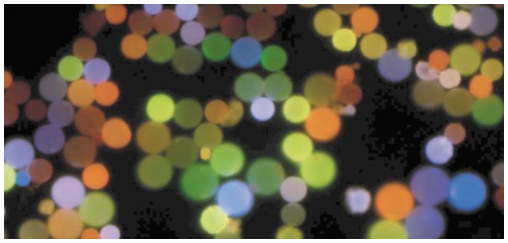
Red Labels

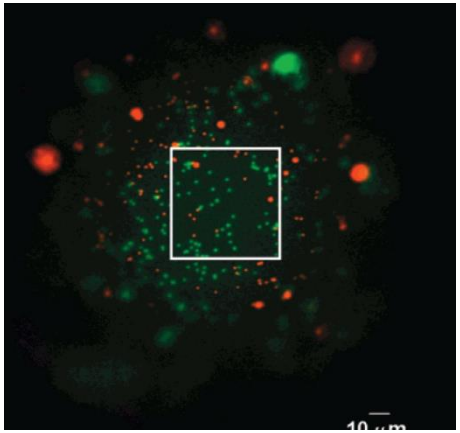
Green Labels

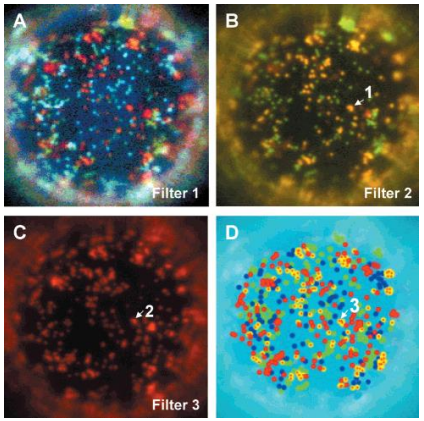
Mix 1 d

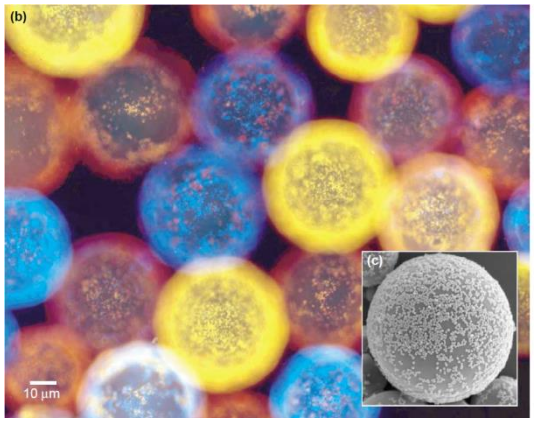
Red Labels

Green Labels

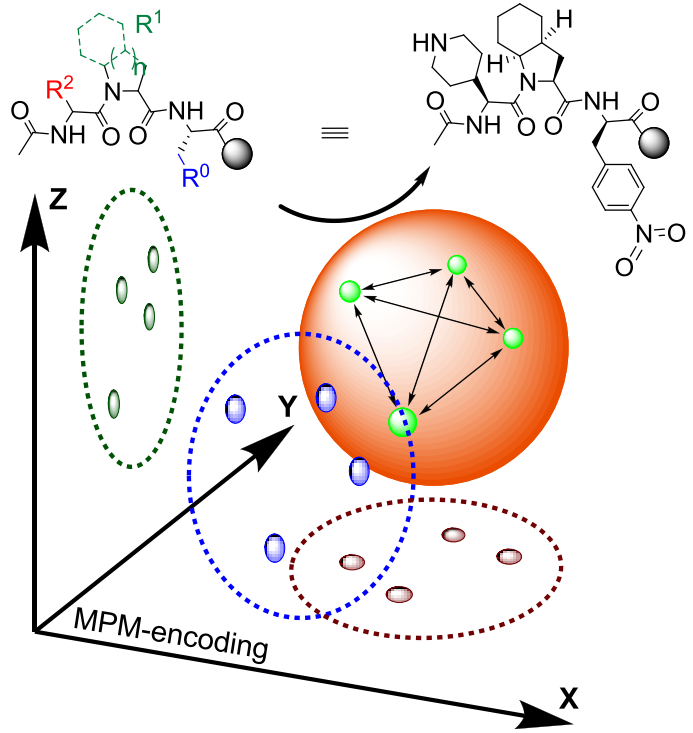








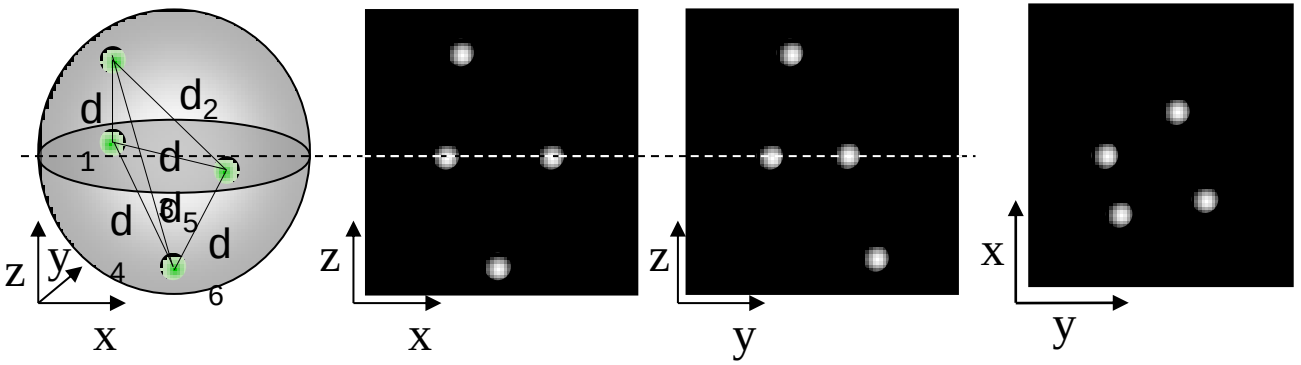
Cross talk
Obstruction of screen

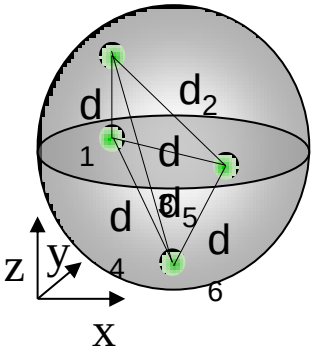


MPM-encoding

500 μm beads and 1 μm resolution :
65449846 unique positions
10^{14}-10^{15} absolute vectors to combine.
Vector combinations are ~infinite

- 3 Orthogonal CCD detectors
  6*2D ….. 3*3D-coordinates
- Inter-particle vectors
  lengths and angles
  orientation independent parameters.
- 3 Particles: 6 vectors, 3 angles
- 4 Particles: 12 vectors, 12 angles etc.





MPM-encoding

Easy to prepare (random code)
Very high coding potential
Optical
Non interfering signal
Resin independent
Stable encoding
Reliable reading
Biochemistry and chemistry
Very fast decoding
Direct structure/activity information

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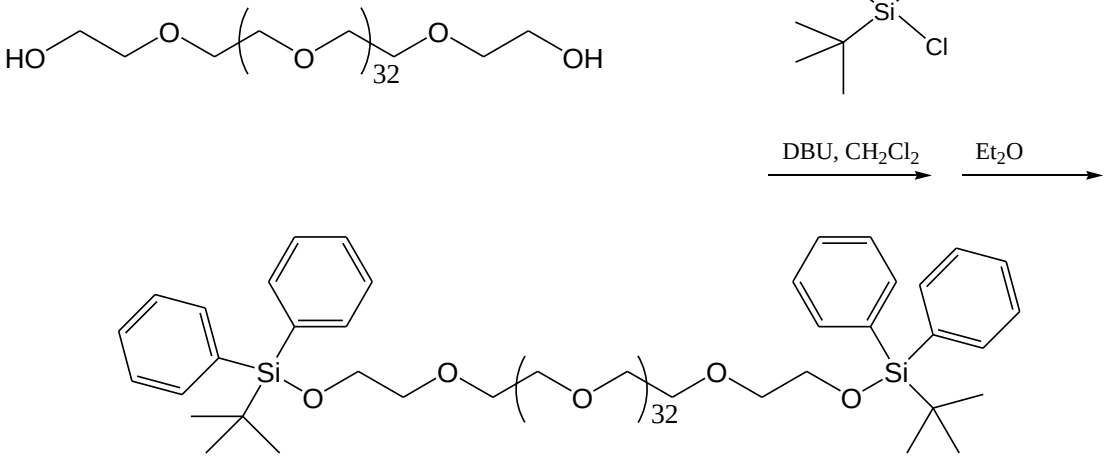
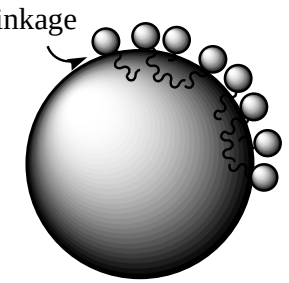
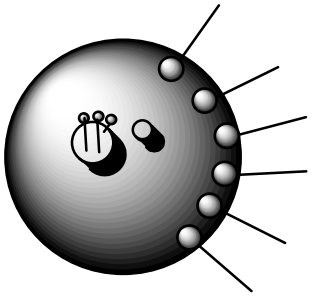


16 mg / 30 g polymerization

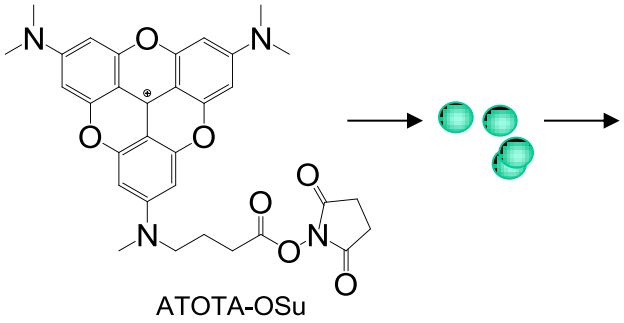


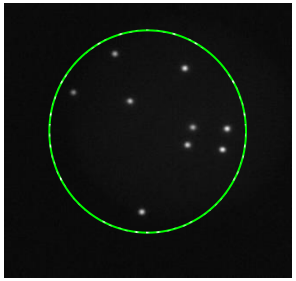
Stable:
- Perfect beads
- Narrow size distribution

Sorbitan monolaurate:
500 mg / 30 g polymerization



Unstable:
- vacuoles
- airbubbles
- irregular shapes
- broad size distribution
- aggregation





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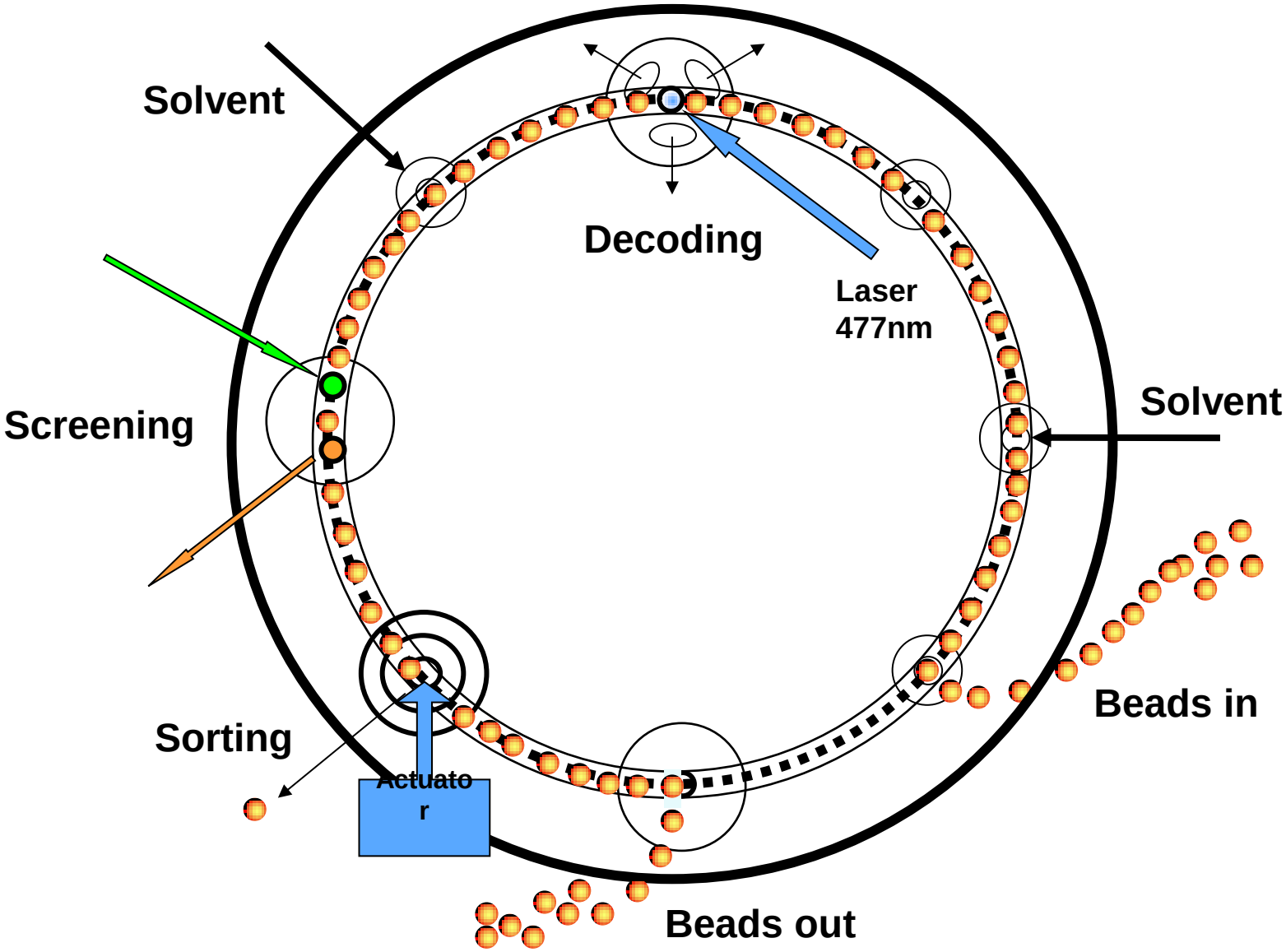

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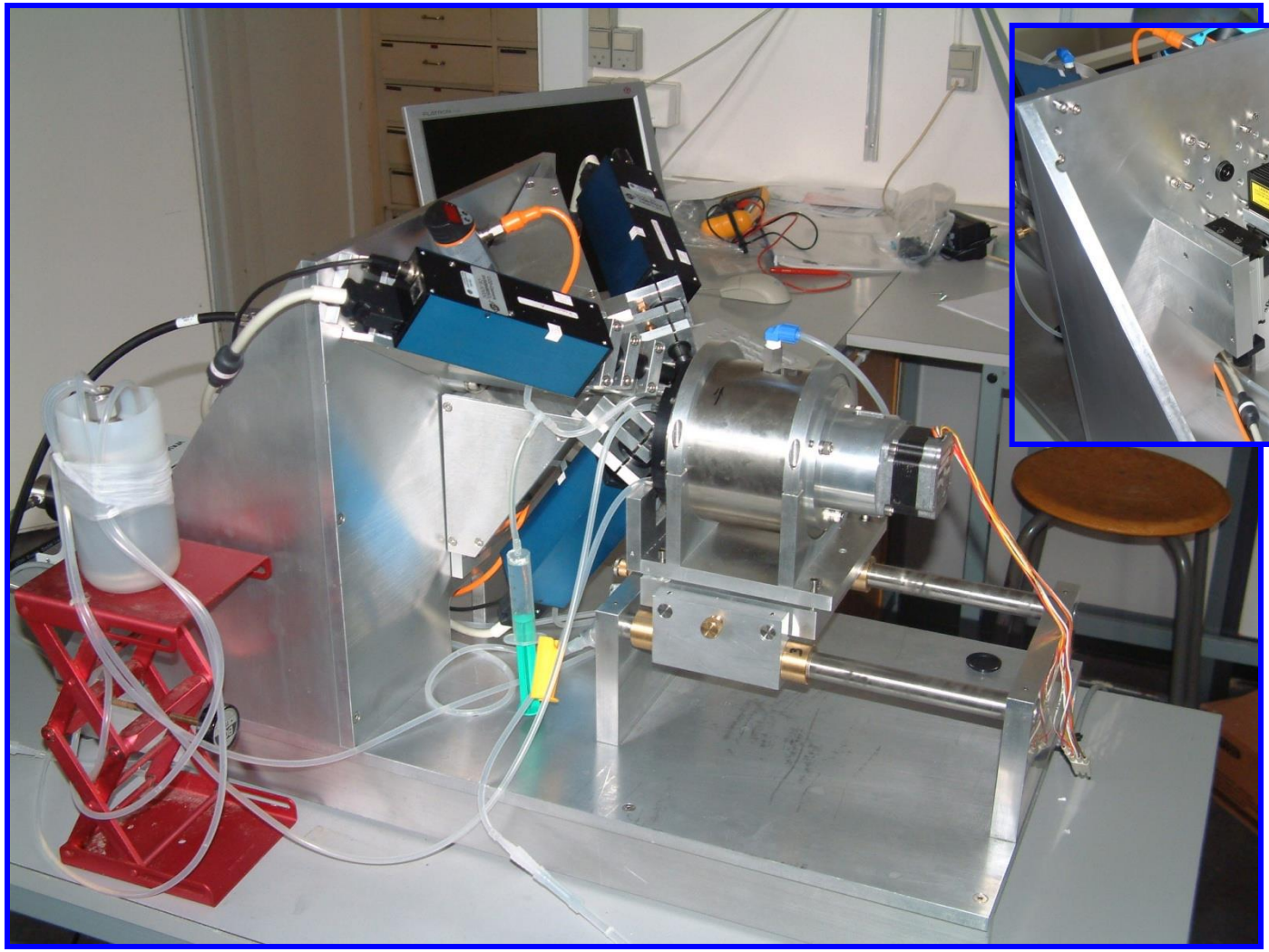
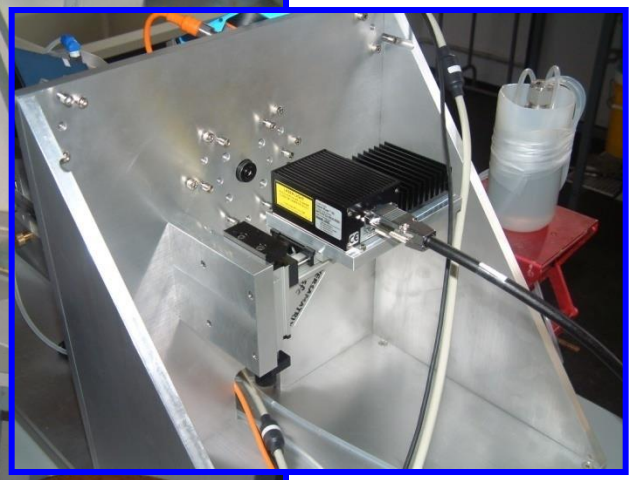




BIFM3 Bead imaging and file management 1280 x 1024 V250206-00 www.staus.dk

Input from user

Output

Split Hits

Present step: 0

Present reactor: 0

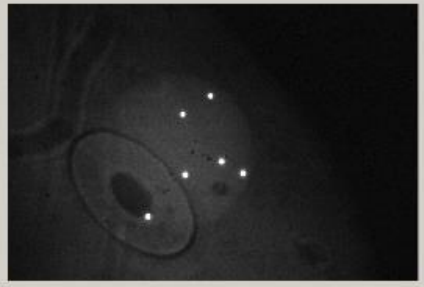
Splits
Hits

PMT Signal 33

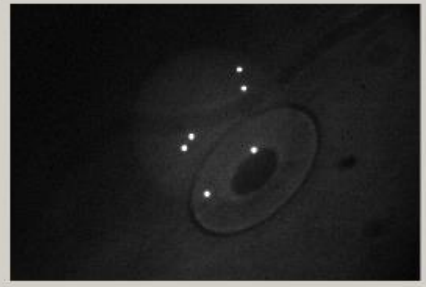
PMT Offset 0

HITS

20-04-2006 12:47:37



Camera A Live 63
H0000000063A.bmp

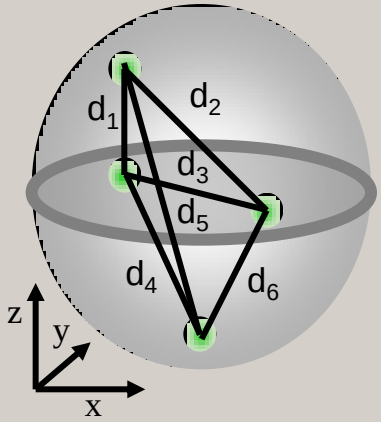


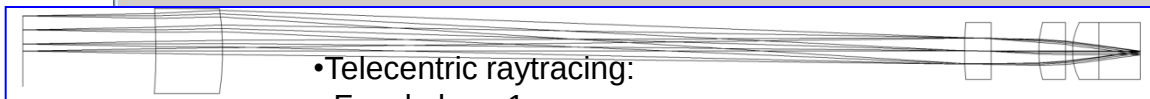
Camera B Live 63
H0000000063B.bmp

T_exposure 90551us

T_pause+HD_Access 787ms

Camera C Live 63
H0000000063C.bmp





•Telecentric raytracing:
• Focal plane 1 mm
• No distortion of image

CCD

Bead

Images x 3 / sec. 1

SYS RUNNING...

RUN STOP PAUSE

Optimal recording speed is 9 images/s with ~50 ms exposure/3 images

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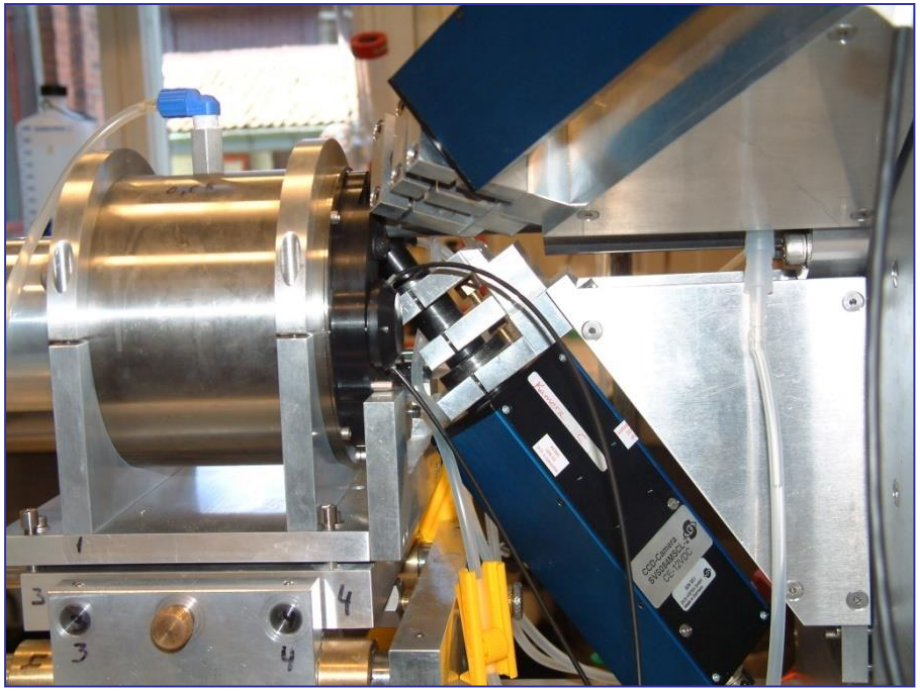
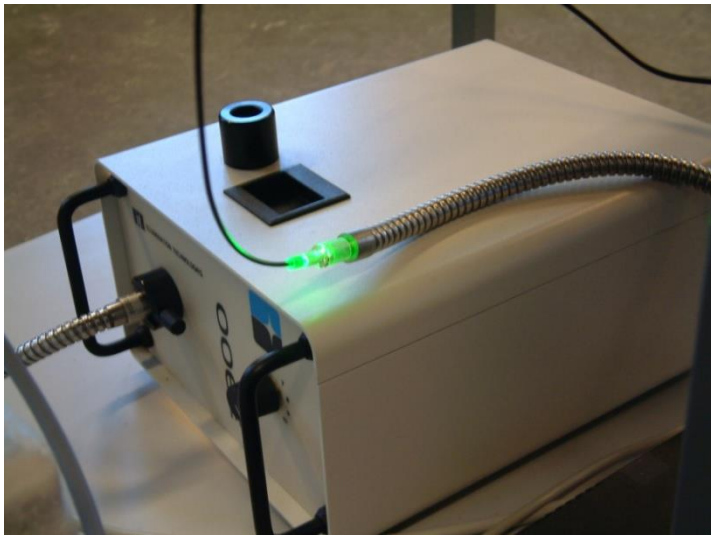
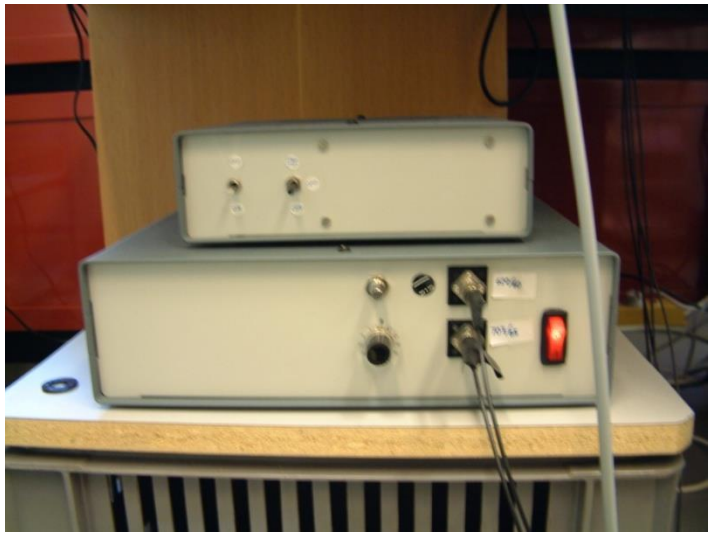
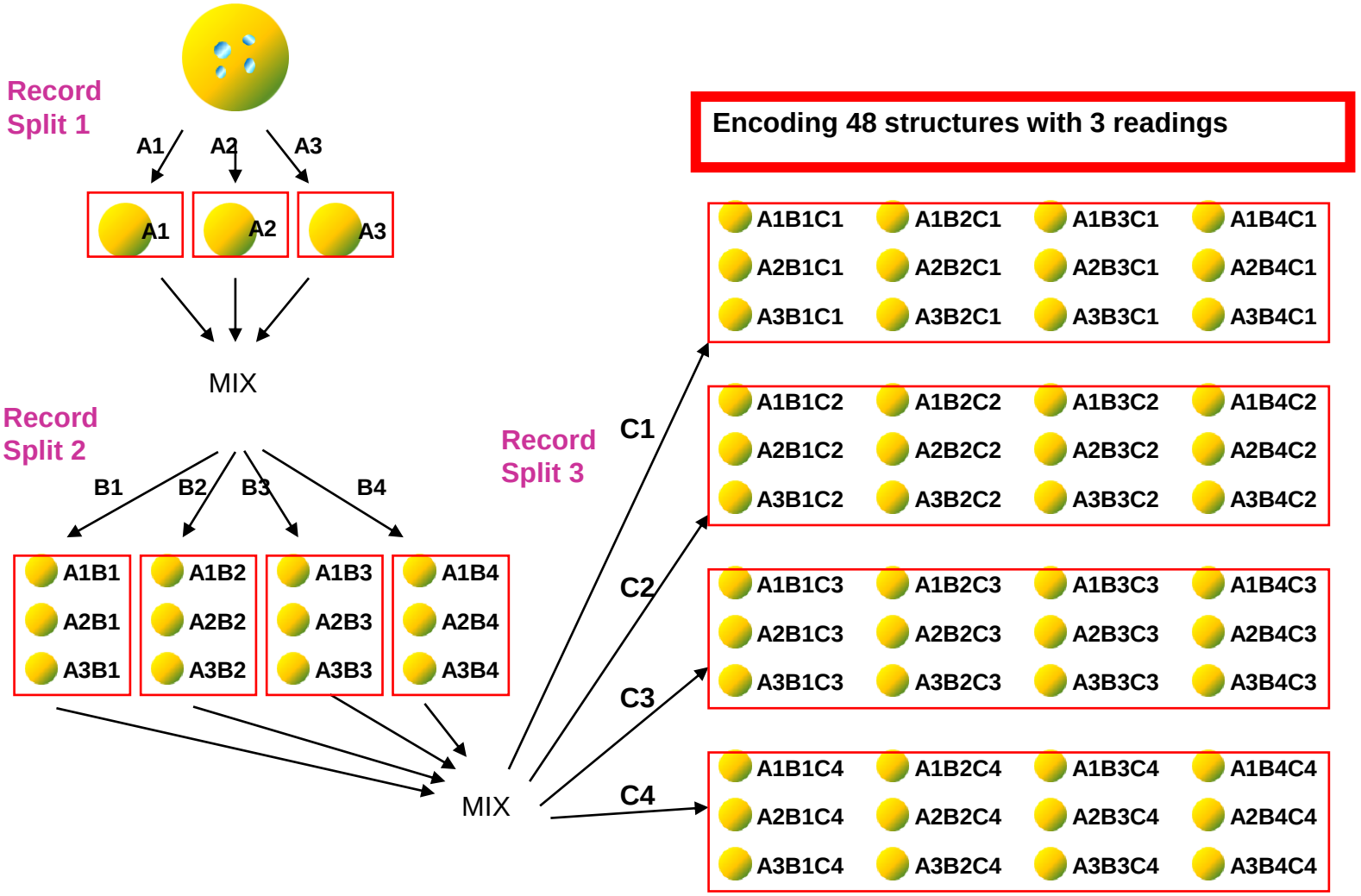






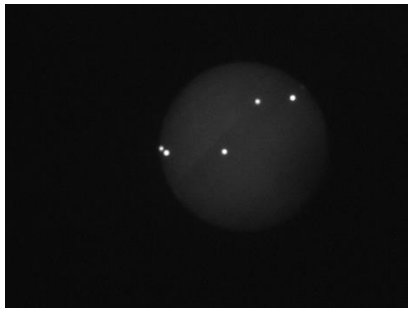
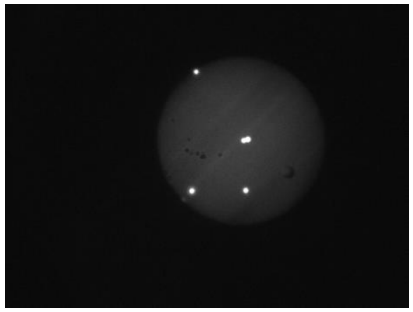
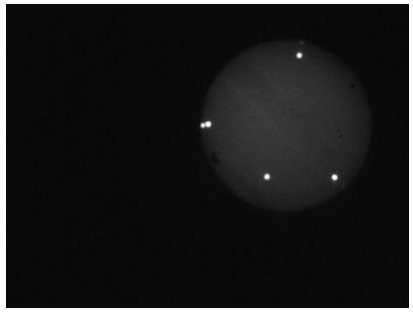
Split Mix library (Furka, Lam) with MPM-encoding



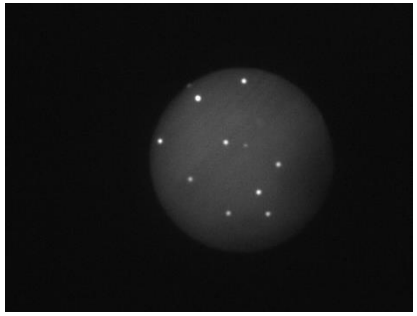
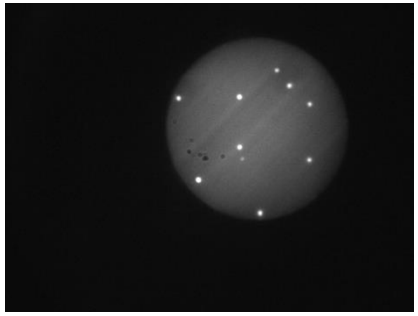
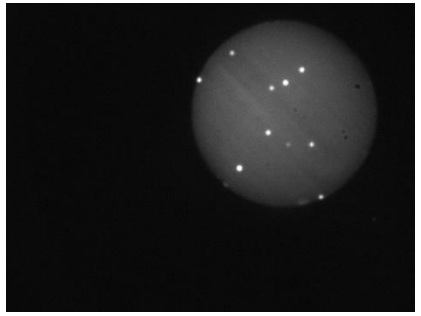
Eight step synthesis: No problem to determine the structure

Selected for maximum background

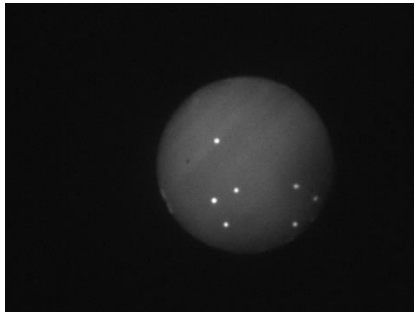
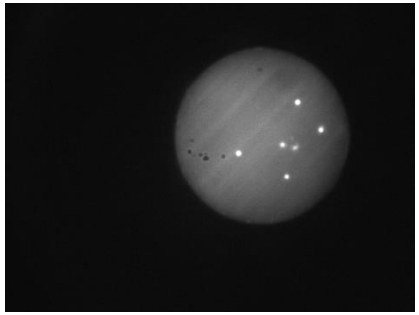
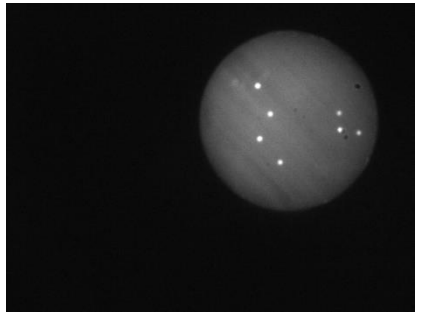
Step

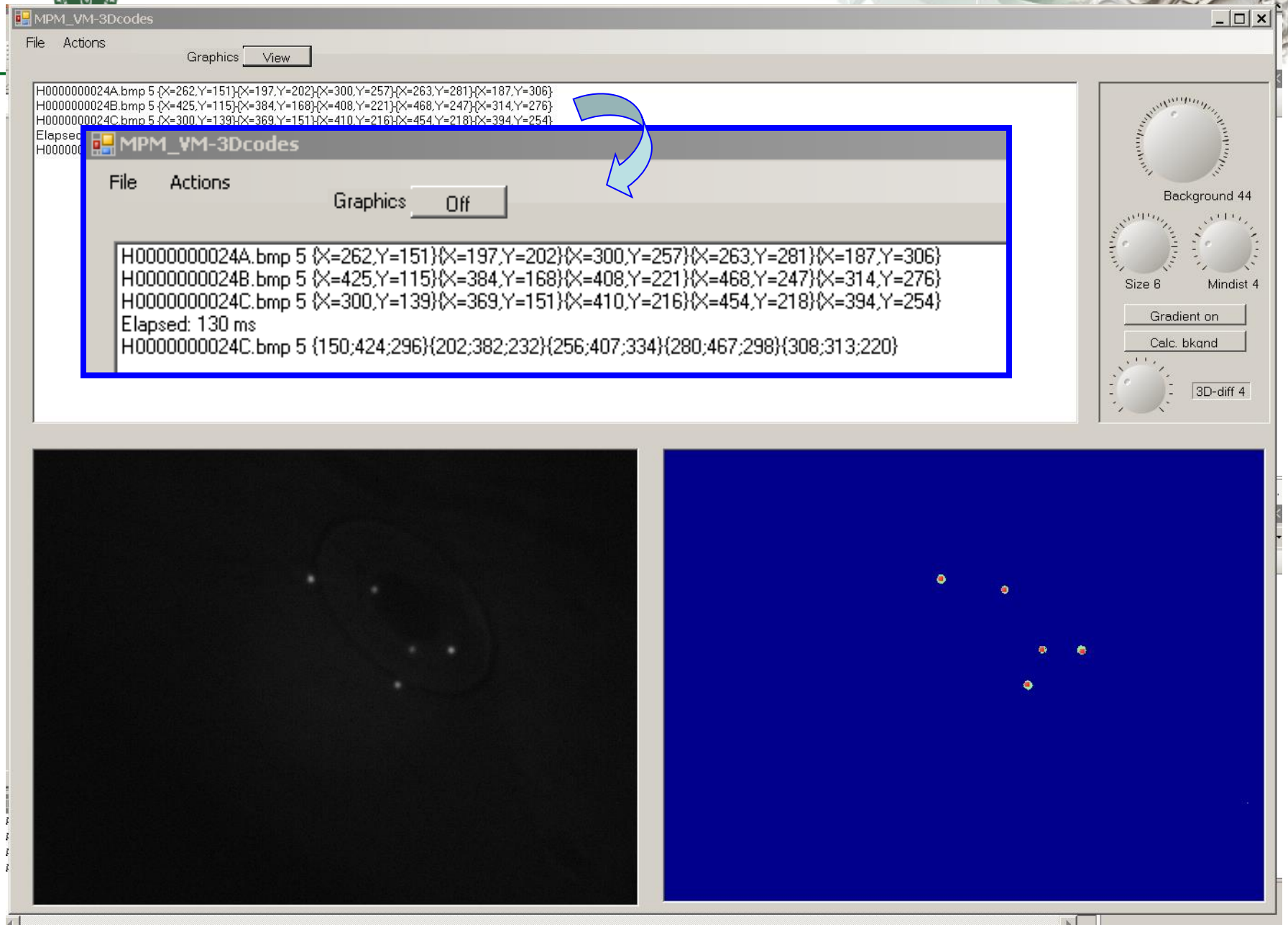
4

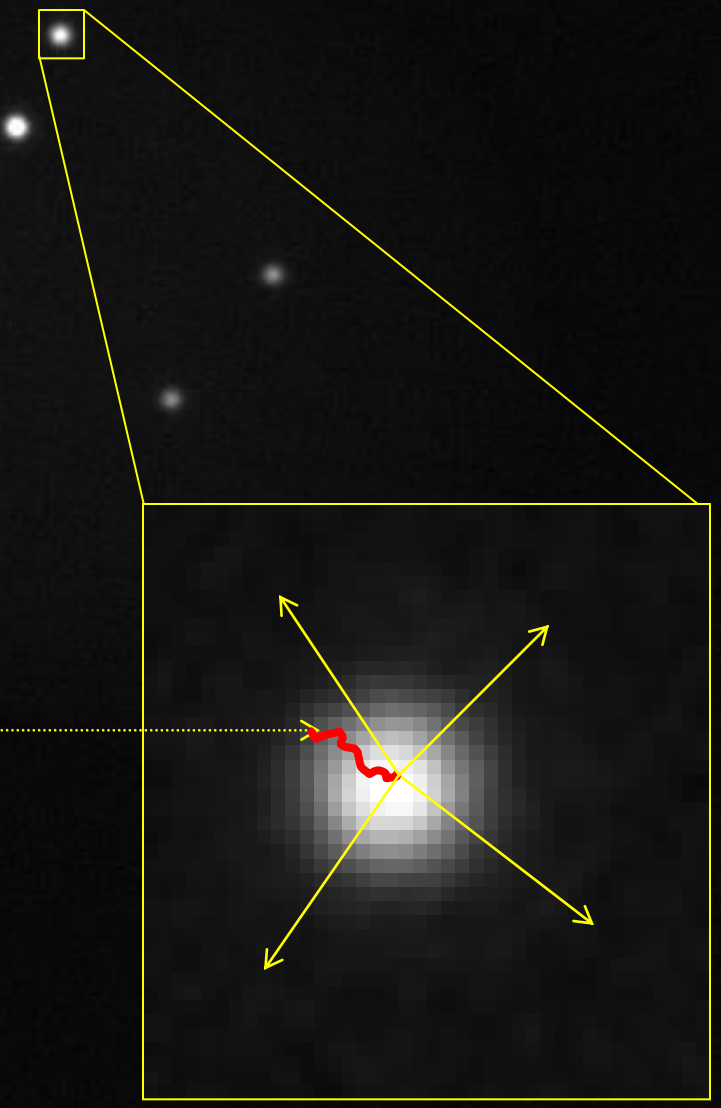
6

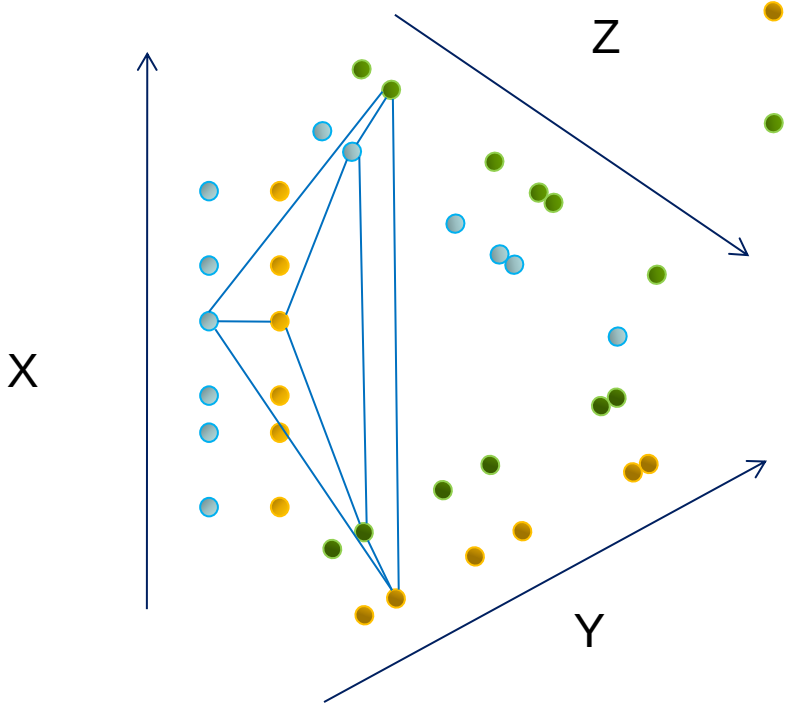
8


MPM_VM-3Dcodes
File
Actions
Graphics
View
H0000000024A.bmp 5 {X=262,Y=151}{X=197,Y=202}{X=300,Y=257}{X=263,Y=281}{X=187,Y=306}
H0000000024B.bmp 5 {X=425,Y=115}{X=384,Y=168}{X=408,Y=221}{X=468,Y=247}{X=314,Y=276}
H0000000024C.bmp 5 {X=300,Y=139}{X=369,Y=151}{X=410,Y=216}{X=454,Y=218}{X=394,Y=254}
Elapsed
H000000
MPM_VM-3Dcodes
File
Actions
Graphics
Off
H0000000024A.bmp 5 {X=262,Y=151}{X=197,Y=202}{X=300,Y=257}{X=263,Y=281}{X=187,Y=306}
H0000000024B.bmp 5 {X=425,Y=115}{X=384,Y=168}{X=408,Y=221}{X=468,Y=247}{X=314,Y=276}
H0000000024C.bmp 5 {X=300,Y=139}{X=369,Y=151}{X=410,Y=216}{X=454,Y=218}{X=394,Y=254}
Elapsed: 130 ms
H0000000024C.bmp 5 {150;424;296}{202;382;232}{256;407;334}{280;467;298}{308;313;220}
Background 44
Size 6
Mindist 4
Gradient on
Calc. bkgnd
3D-diff 4

1) Over background ?
2) Over local background ?
3) Surrounding spots ?
4) Locate max intensity
5) Go repeatedly to surrounding sphere
6) A gradient in all directions confirmed



The 3D matrix from 2D images



- Image 1
- Image 2
- Image 3

3 Images ~ 6 Point collections

Select relation e.g. 1 < 2 = 3

Least square fit alignment

Correlate all points

Reconstruct missing point or remove

Least squares fit

Correlate all points

Calculate average coordinates

X1, Y1, Z1

X2, Y2, Z2

X3, Y3, Z3

X3, Y3, Z3

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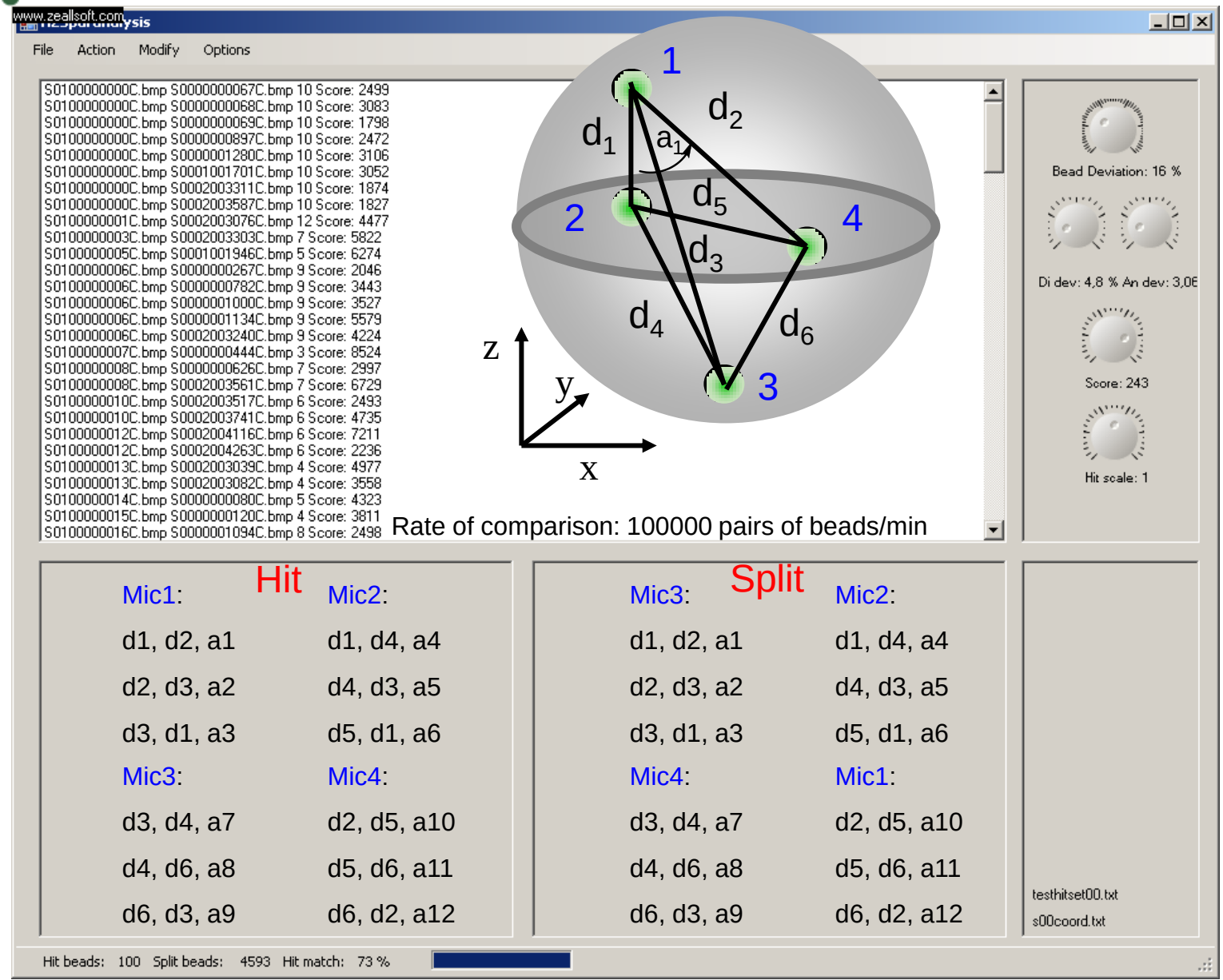

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H2Sparanalysis

File Action Modify \coordmpmgl10-3.txt \coordmpmgl10-4.txt

```
H0000000006C.bmp 4 234;263;316 249;284;333 256;385;444 292;322;366
S0000000015C.bmp 4 158;270;320 226;288;242 238;350;222 245;376;207
S0000000119C.bmp 4 159;270;318 228;287;240 240;349;220 242;349;206
H0000000006C.bmp S0000000015C.bmp Score: 6521
H0000000006C.bmp S0000000015C.bmp Min distance: 13,0322817425155
H0000000006C.bmp S0000000119C.bmp Score: 6521
H0000000006C.bmp S0000000119C.bmp Min distance: 58,4602848022095

H0000000007C.bmp 4 166;373;308 254;472;316 290;315;390 308;401;350
S0000000110C.bmp 4 254;414;265 269;382;353 288;260;266 311;304;388
H0000000007C.bmp S0000000110C.bmp Score: 10963
H0000000007C.bmp S0000000110C.bmp Min distance: 28,0669480656057

H0000000039C.bmp 4 200;298;289 216;290;305 286;308;297 334;290;338
S0000000067C.bmp 4 186;422;240 214;332;320 230;392;280 232;324;334
H0000000039C.bmp S0000000067C.bmp Score: 6521
H0000000039C.bmp S0000000067C.bmp Min distance: 28,8250020357989

H0000000066C.bmp 4 162;416;354 172;387;290 228;454;308 240;296;340
S0000000070C.bmp 4 218;338;173 262;266;328 264;266;194 298;326;210
H0000000066C.bmp S0000000070C.bmp Score: 6521
H0000000066C.bmp S0000000070C.bmp Min distance: 76,9887245544687

H0000000074C.bmp 5 142;325;273 152;398;279 168;208;368 182;436;350 268;408;270
S0000000127C.bmp 5 208;456;442 240;352;388 314;497;432 328;466;308 346;436;452
H0000000074C.bmp S0000000127C.bmp Score: 6148
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```
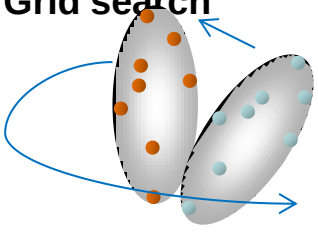

Secondary matching:

Eliptic fitting

MonteCarlo-Simulated Anneling

Grid search



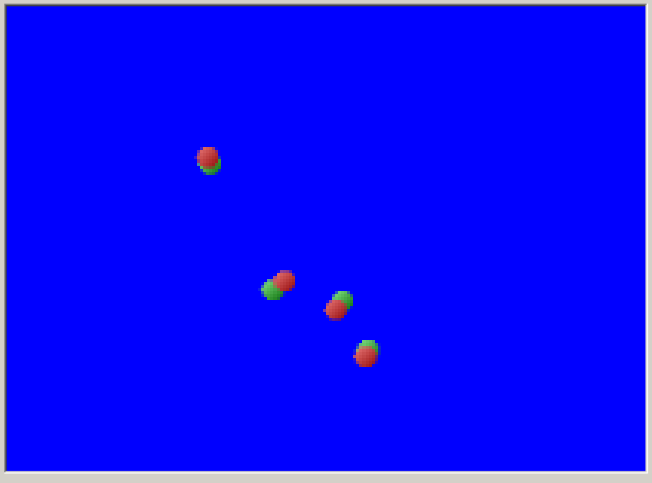
Long axis
Short axis
Perpendicular axis

Bead Deviation: 9 %

Dist Deviation: 1 %

Angle Deviation: 1 %

Score: 4879

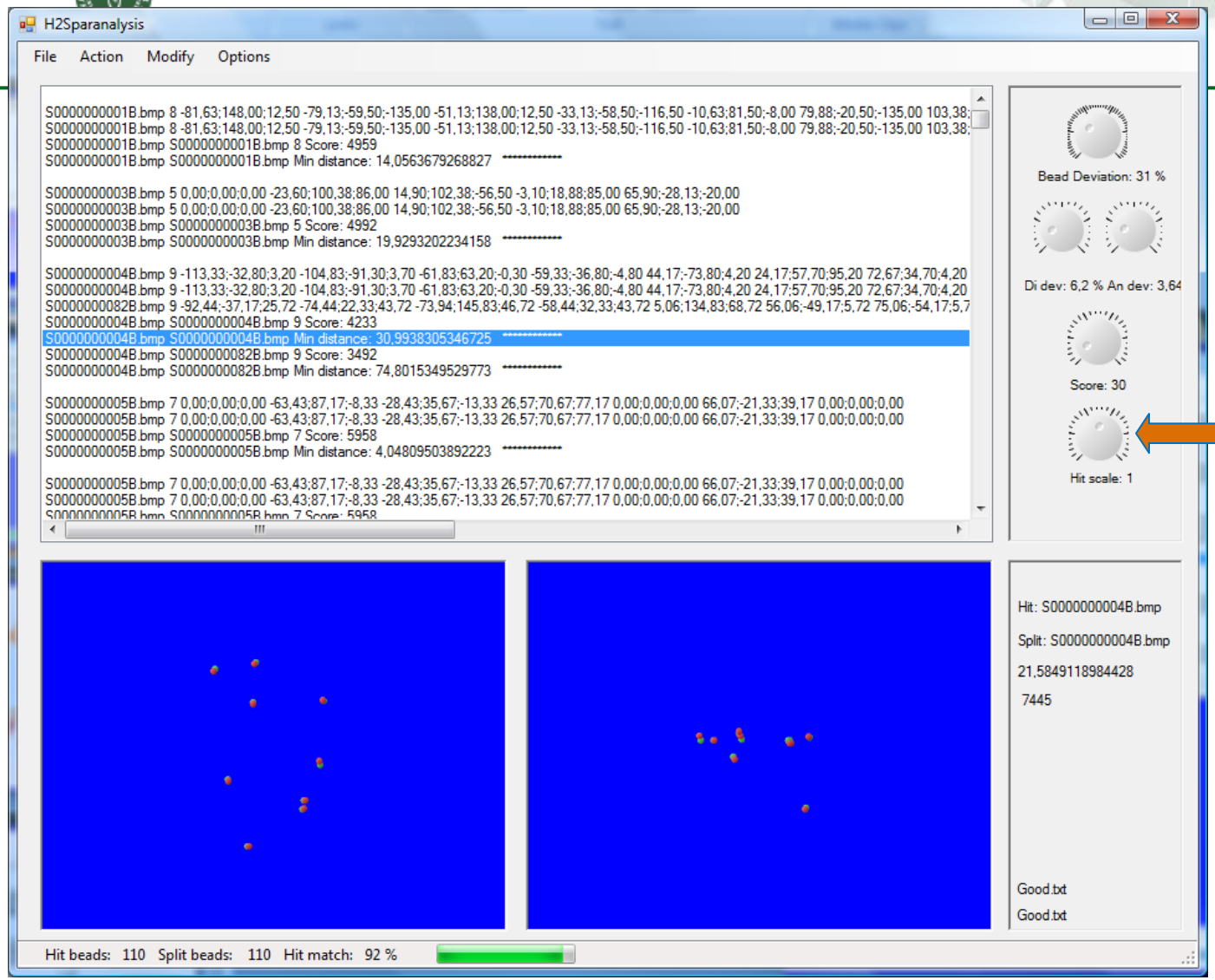


Hit: H0000000968C.bmp

Split: S0000000222C.bmp

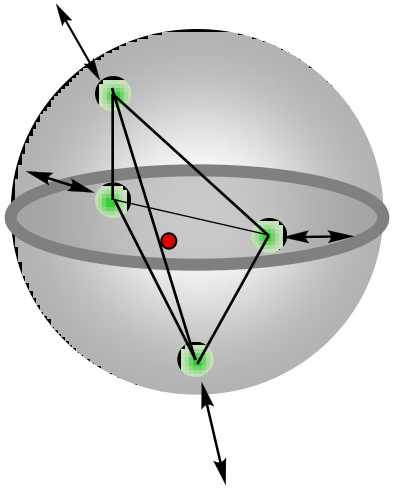
24,815966741487

10963



Polarity ?
Loading ?
Solvent / solute

Observation:
No swelling
Distortions



Neither swelling nor RI seem to be a problem and H_2O remains the best liquid for decoding on PEGA resin

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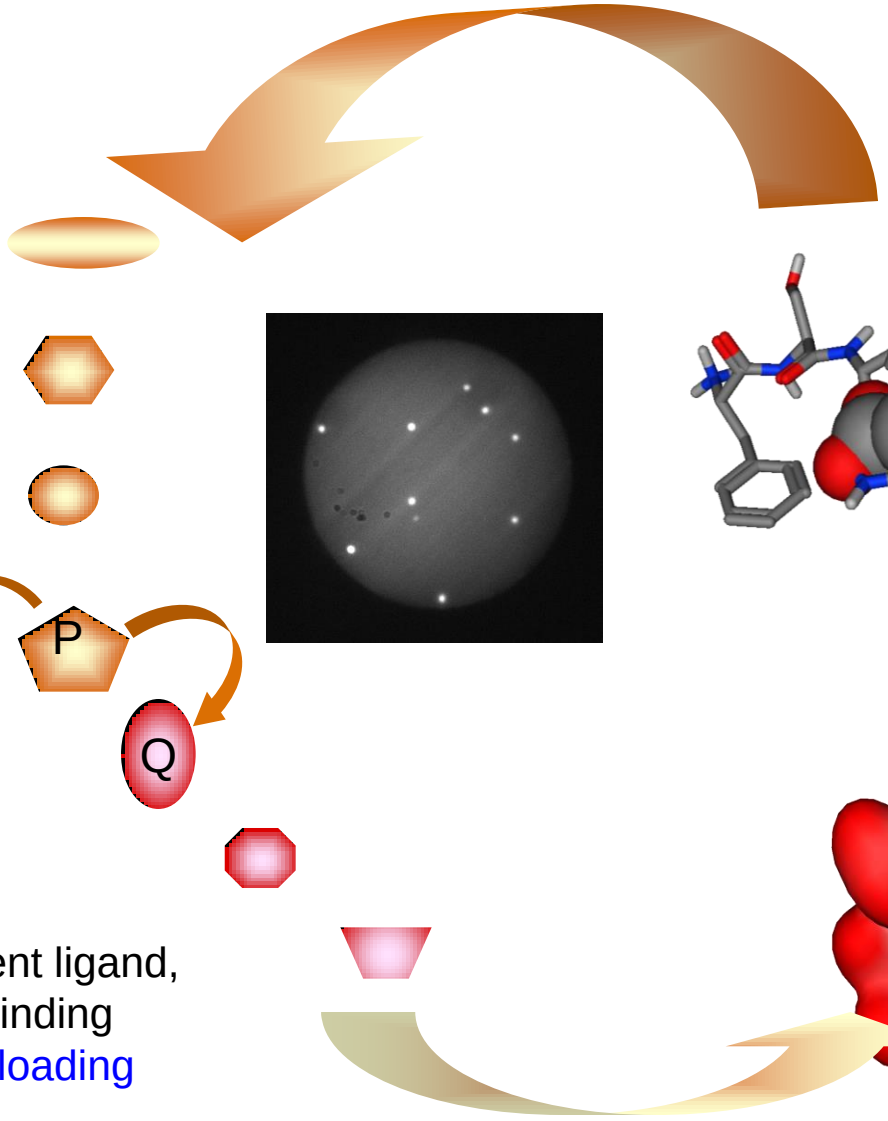
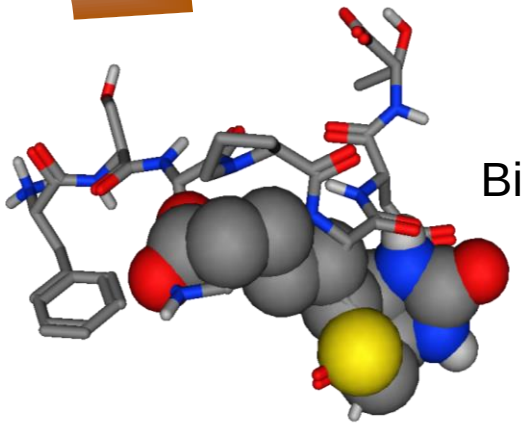
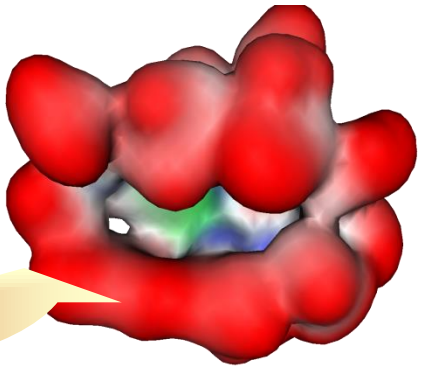
R_2	R_1	R_0
val	Aze	Gln
His	Pro	gln
his	pro	NO_2Phe
Tha	NmeA	NO_2phe
3-Pya	NipCA	Cit
4-Pya	PipCA	3CNPhe
4-PipG	Oic	4CNPhe





Biotin

Avidin



Multivalent ligand,
Strong binding
Reduce loading

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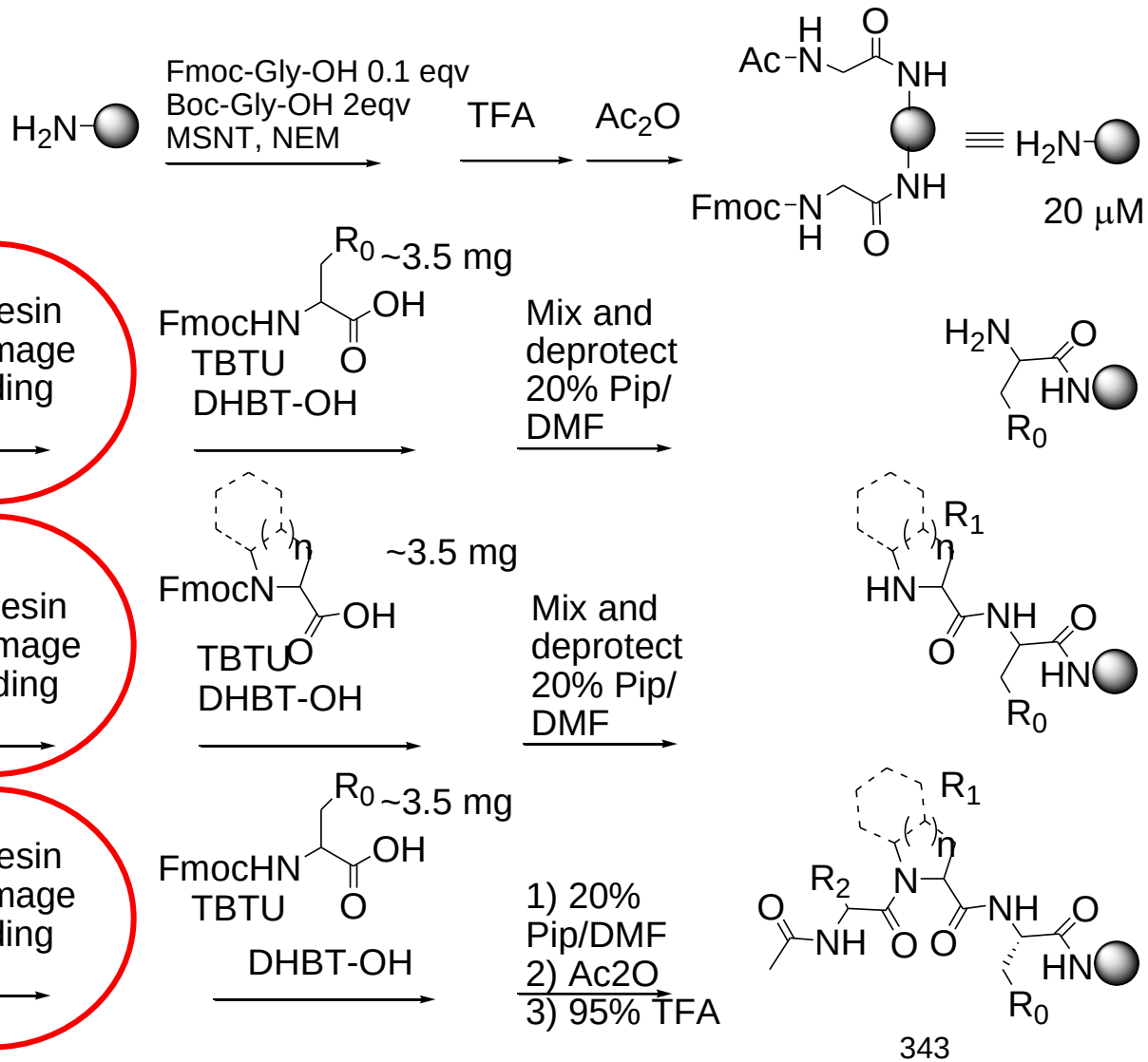
100 mg amino acid derivatives totally



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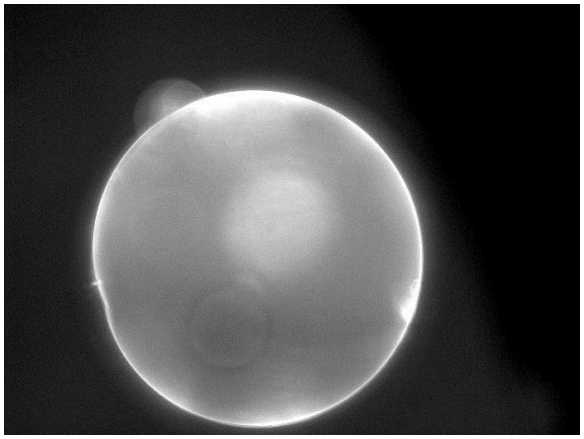
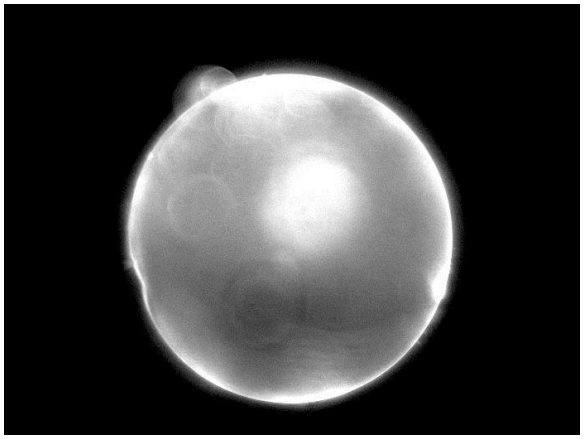
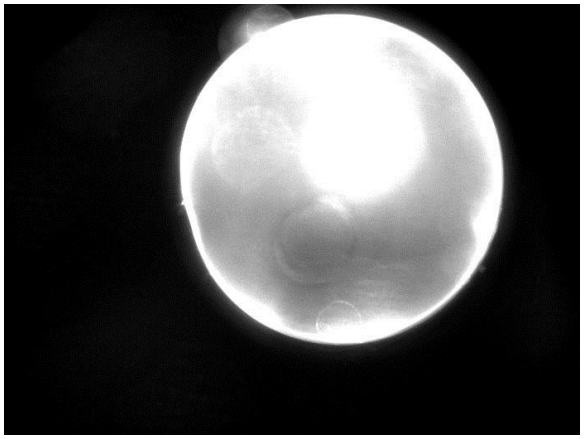
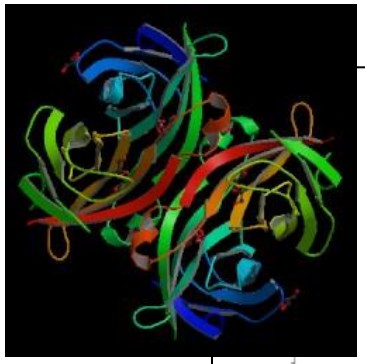
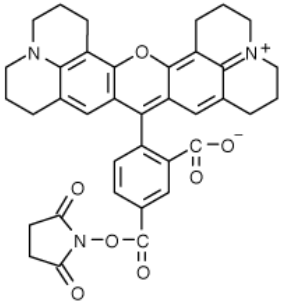


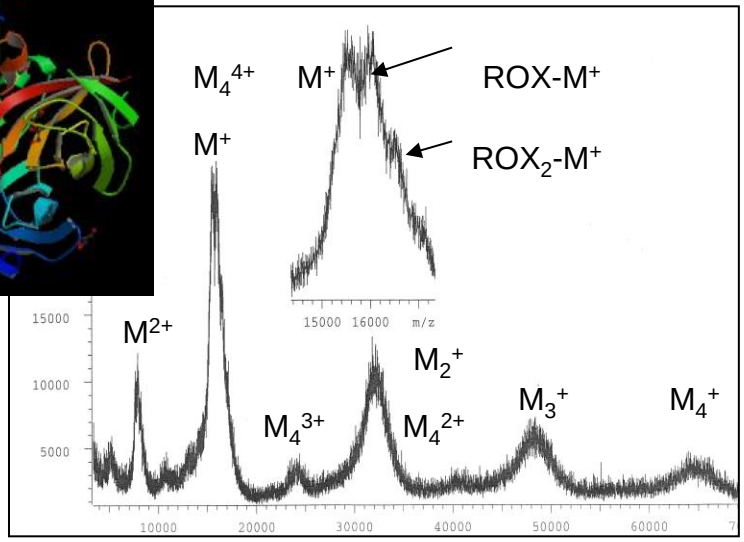




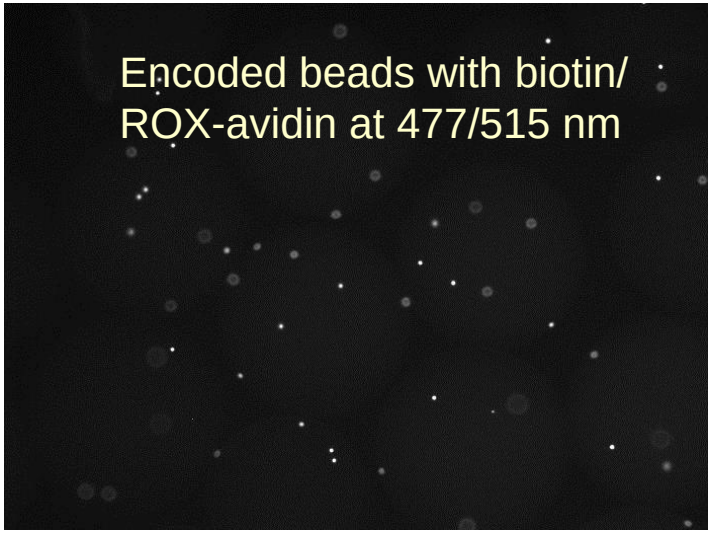




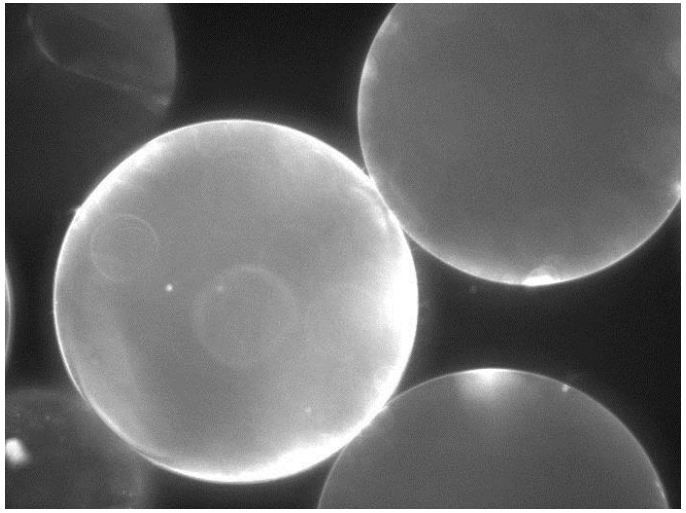
24h

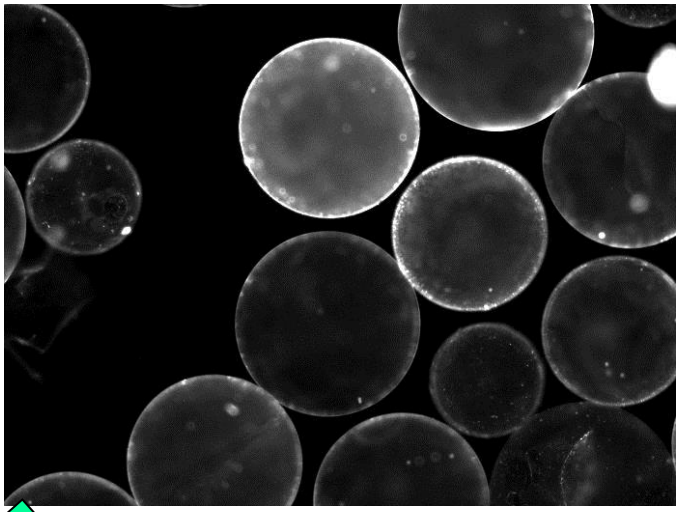


72h

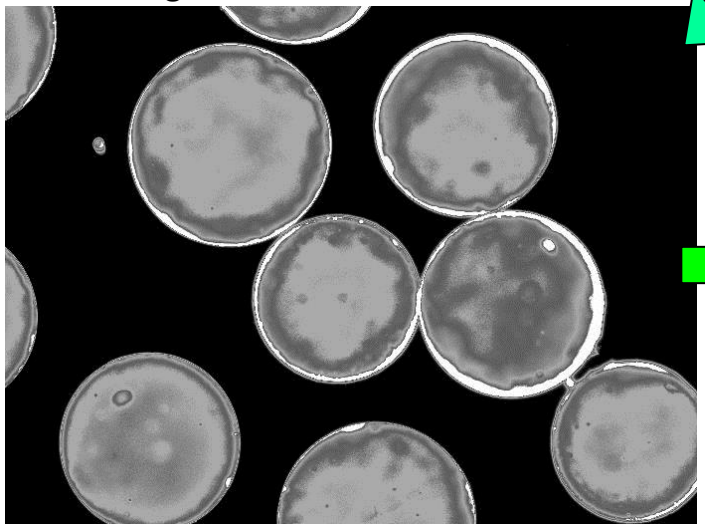


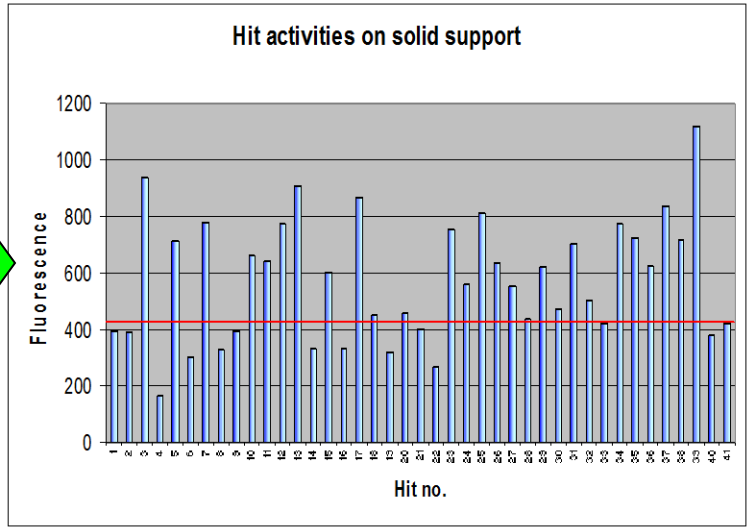
Hits on library background /BSA 2 mM





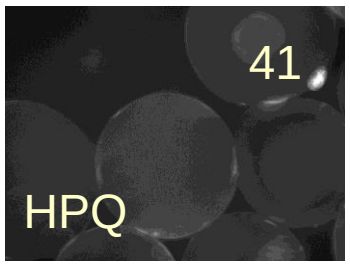
Decoding

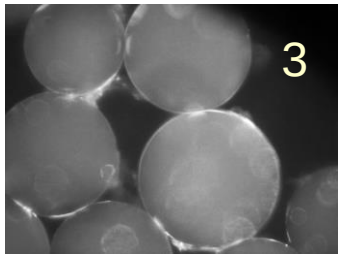


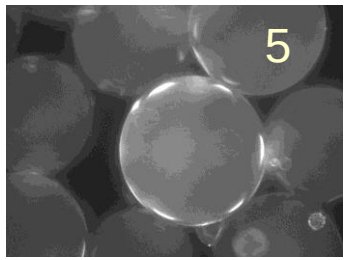


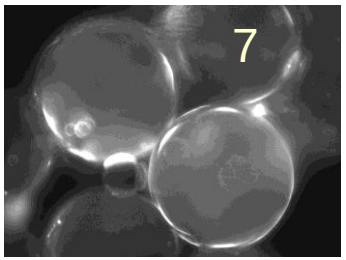
30 beads decoded giving 29 structures

Resynthesized peptides

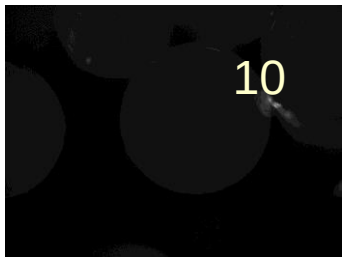


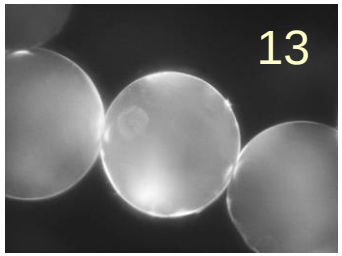


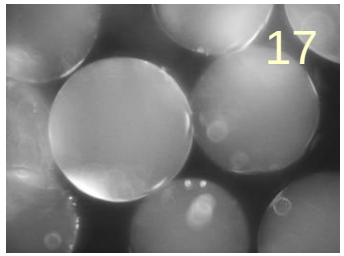


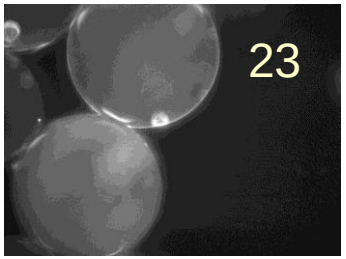


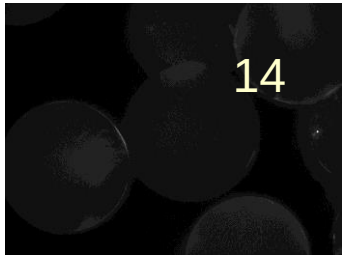
Less active

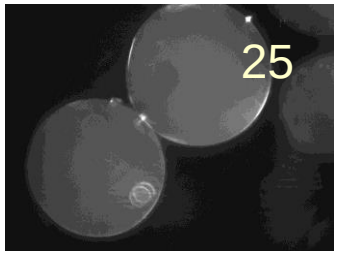


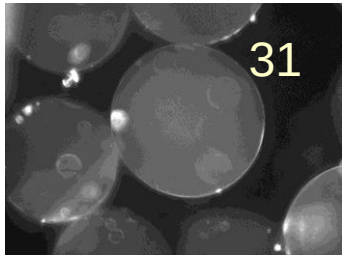


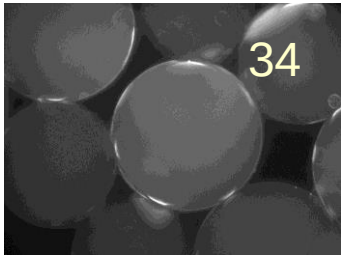


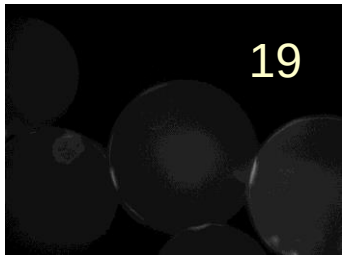


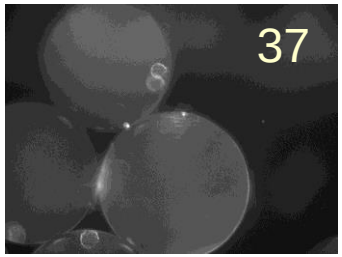


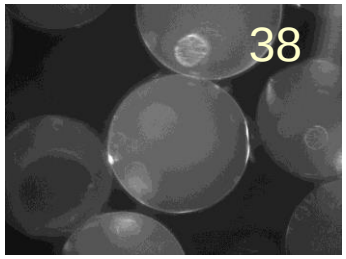


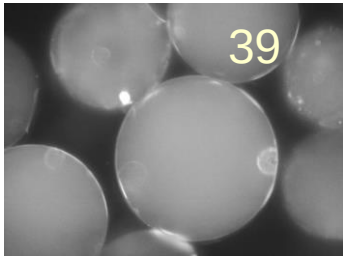












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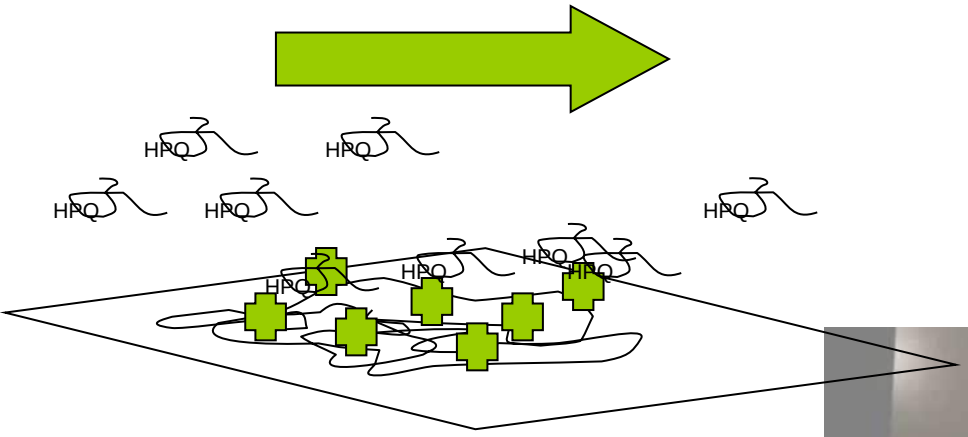
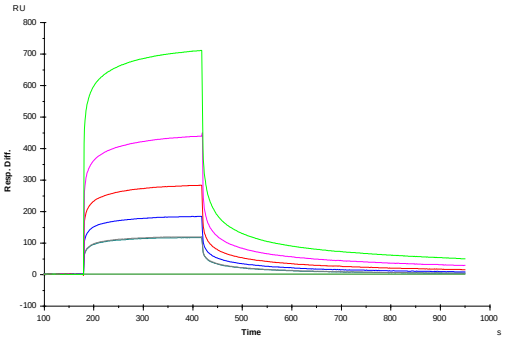
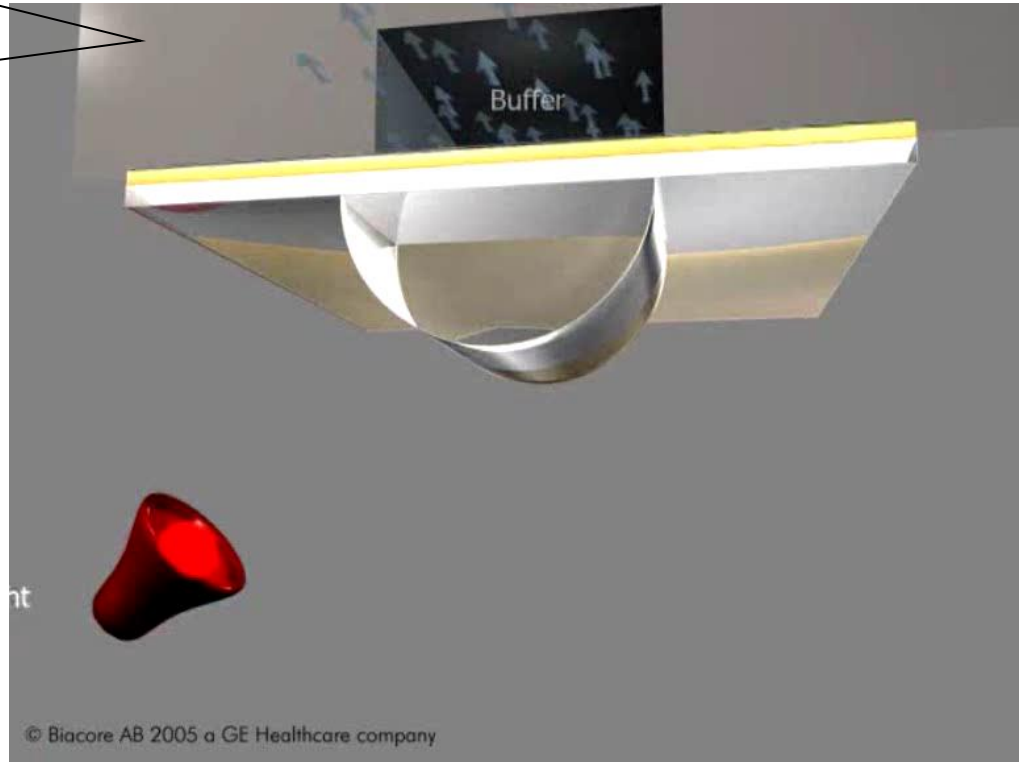


- Avidin immobilized on CM5
- Tetravalent binding
- Lipophilic interactions





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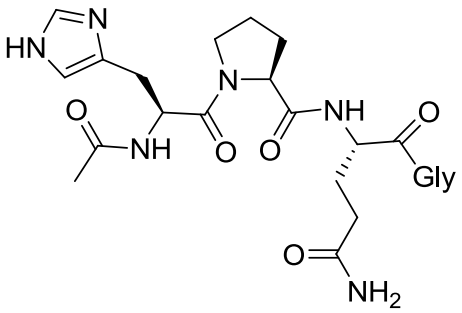
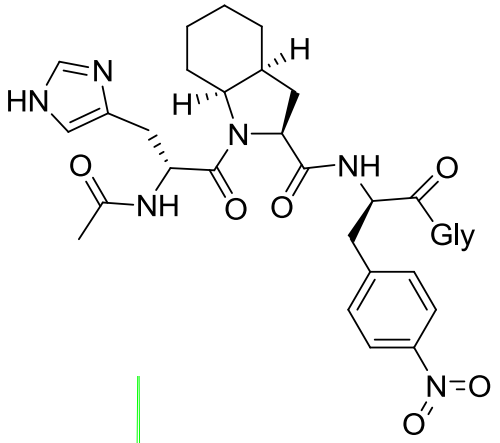
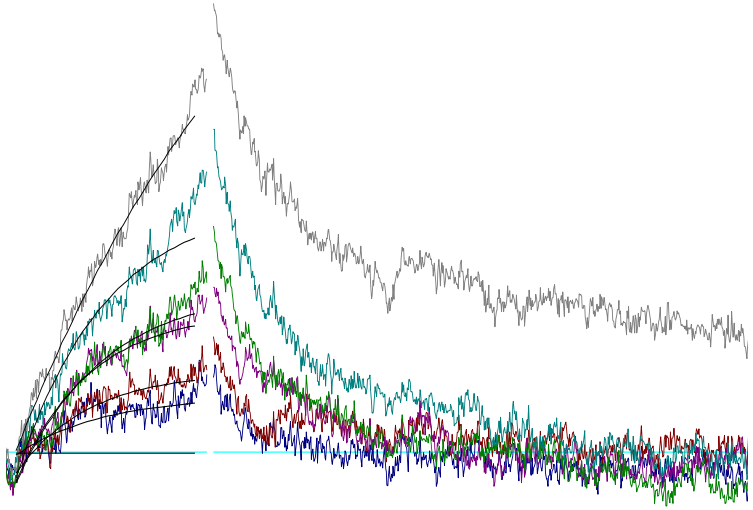
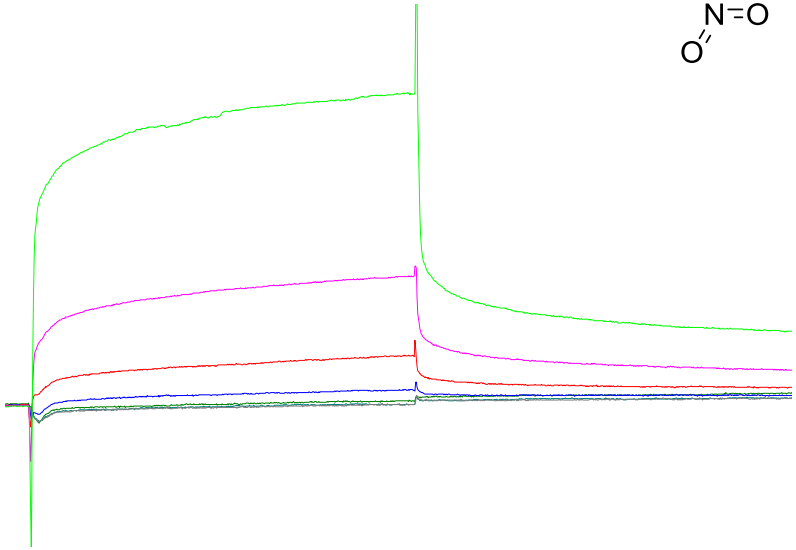


HPQ:

hOn:







$K_D = 1.21 * 10^{-2}$ (M)

$K_D = 1.4 * 10^{-5}$ (M)

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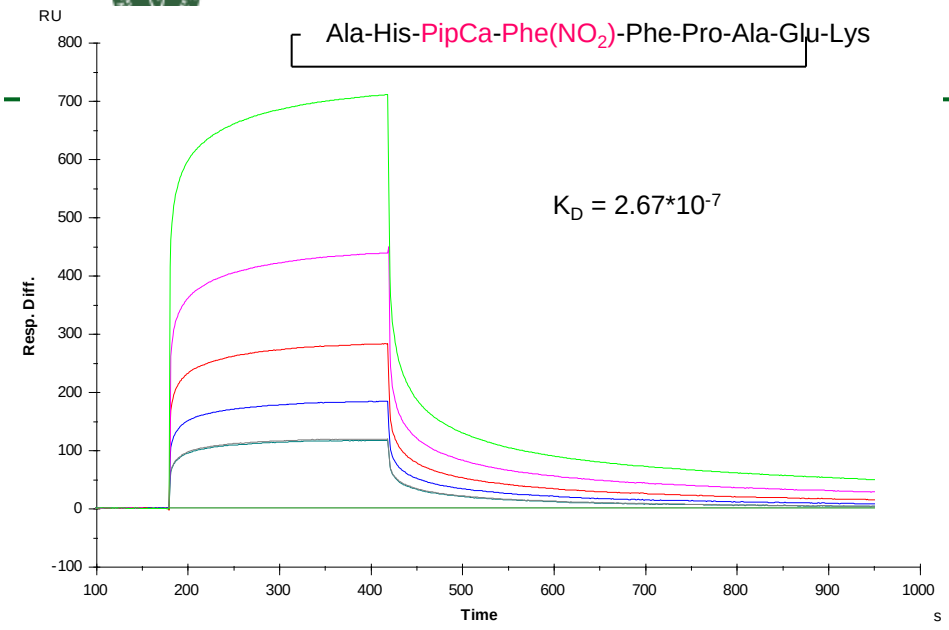
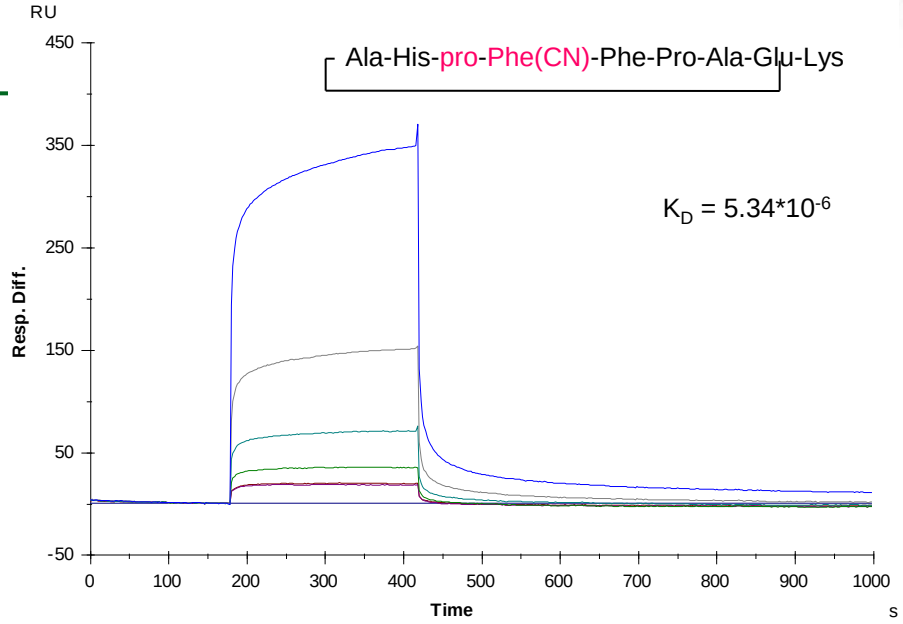
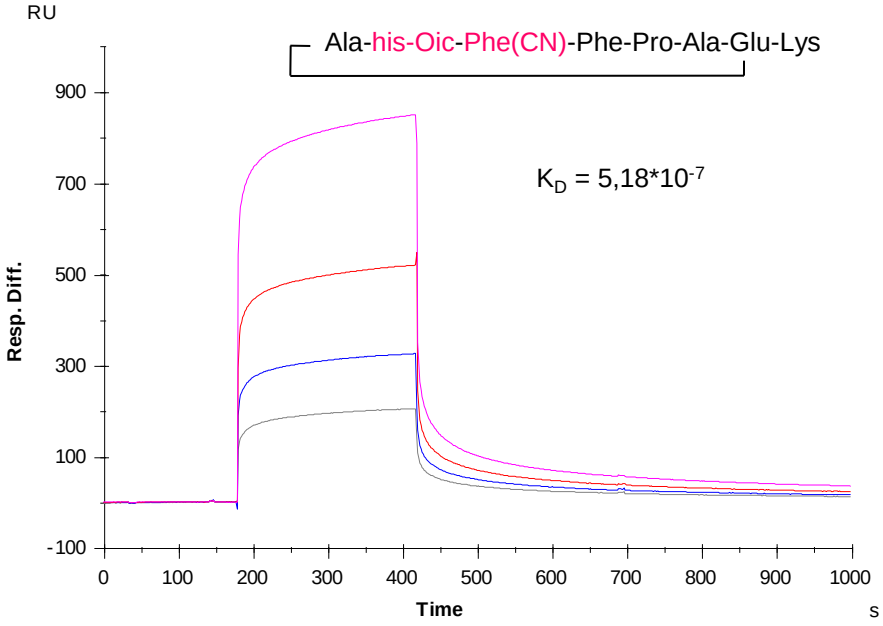
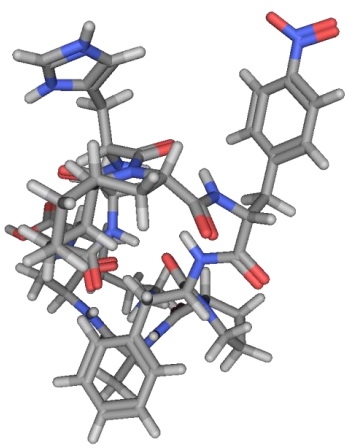
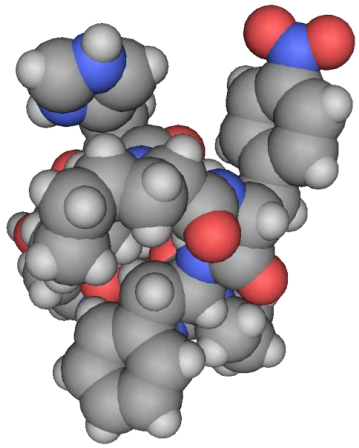
Structure				ESI-MS m/z (Calc.)	Kd /M	
his	Aze	Phe(4-CN)	Gly	510.2091 (510.2101)	2,33E-06	
His	Pro	Gln	Gly	480.1641 (480.2206)	1,21E-02	
his	Oic	phe(4-NO2)	Gly	598.2611 (598.2625)	1,42E-05	
Tha	NipCa	phe(4-NO2)	Gly	574.1982 (574.1971)	5,00E-05	
his	Pro	phe(4-NO2)	Gly	544.2144 (544.2156)	4,60E-05	
Tha	NmeA	Phe(4-CN)	Gly	512.2263 (512.2258)	6,99E-06	
his	Aze	phe(4-NO2)	Gly	530.2002 (530.2000)	3,72E-06	
Tha	Aze	Gln	Gly	482.1696 (482.1710)	2,13E-05	
4-PyA	Aze	phe(4-NO2)	Gly	541.2048 (541.2047)	7,05E-05	
His	pro	Phe(4-CN)	Gly	524.2251 (524.2258)	2,08E-05	
His	Oic	Phe(4-CN)	Gly	578.2719 (578.2727)	6,60E-06	
4PipG	Oic	phe(4-NO2)	Gly	601.2972 (601.2986)	8,90E-06	
His	NmeA	gln	Gly	468.2199 (468.2207)	2,70E-05	
Tha	Pro	phe(4-NO2)	Gly	560.1815 (560.1815)	8,73E-05	
Cyclic: Binding residues						Structure
his	Oic	Phe(4-NO2)		562.7830 (562.7803)	2,44E-06	*c* -Ala-his-Oic-Phe(NO2)-Phe-Pro-Ala-Glu-Lys
his	Oic	phe(4-NO2)		562.7805 (562.7803)	1,78E-06	*c* -Ala-his-Oic-phe(NO2)-Phe-Pro-Ala-Glu-Lys
His	pro	Phe(4-CN)		525.7618 (525.7617)	5,34E-06	*c* -Ala-His-pro-Phe(4-CN)-Phe-Pro-Ala-Glu-Lys
His	PipCA	Phe(4-NO2)		542.79 (542.7644)	2,67E-07	*c* -Ala-His-PipCa-Phe(4-NO2)-Phe-Pro-Ala-Glu-Lys
his	Oic	Phe(4-CN)		552.80 (552.7852)	5,18E-07	*c* -Ala-his-Oic-Phe(CN)-Phe-Pro-Ala-Glu-Lys
His	Pro	Gln		503.7885 (503.7591)	6,70E-02	*c* -Ala-His-Pro-Gln-Phe-Pro-Ala-Glu-Lys
						c -: Cyclization between Glu side-chain and *N*-terminal Ala







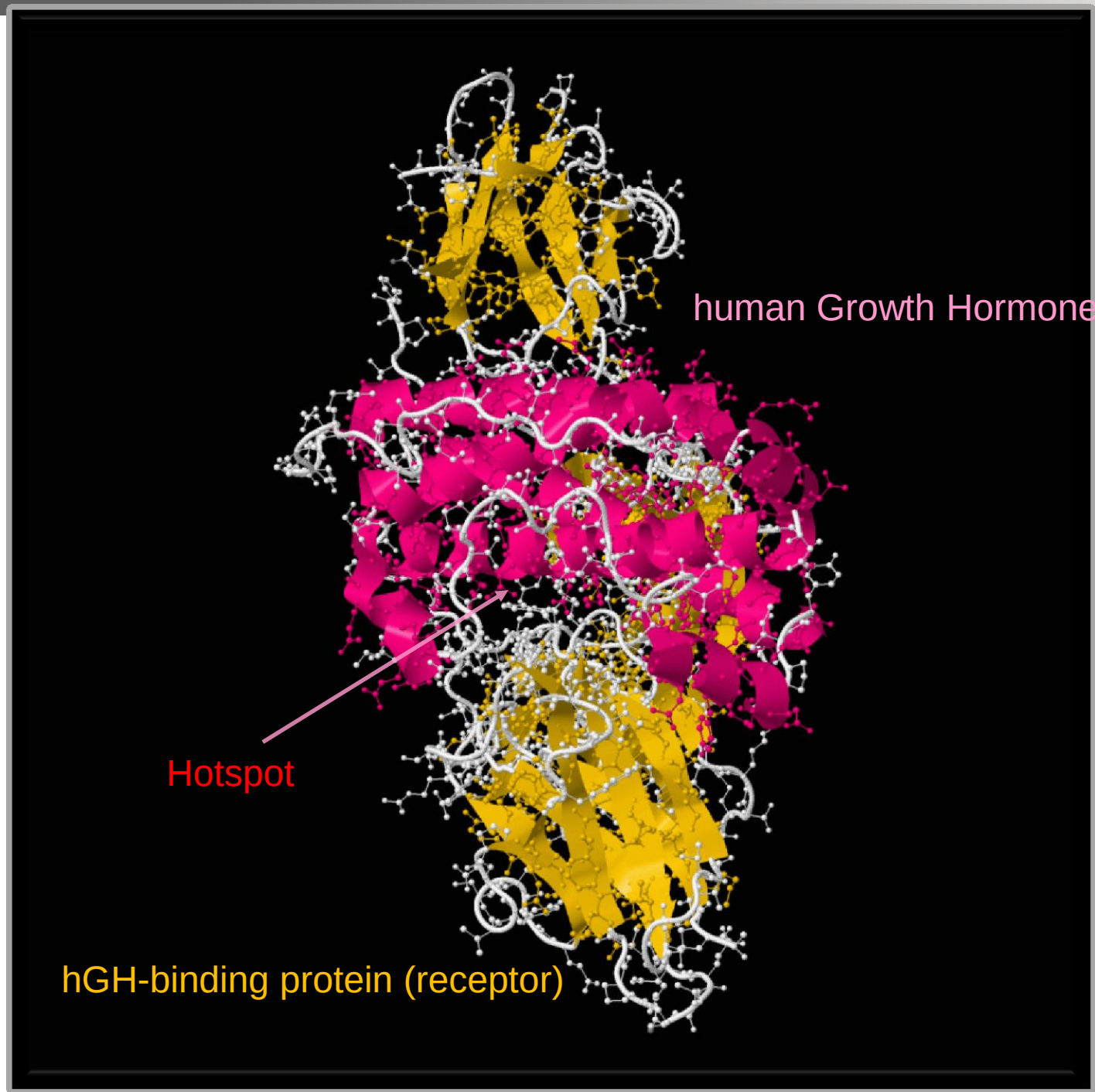




Binding is not additive
Binding is not increased significantly by cyclization

Novonordisk A/S
hGH production and
Purification (Protein A)

Jakob E. Rasmussen



Ligands for human growth hormone (hGH)

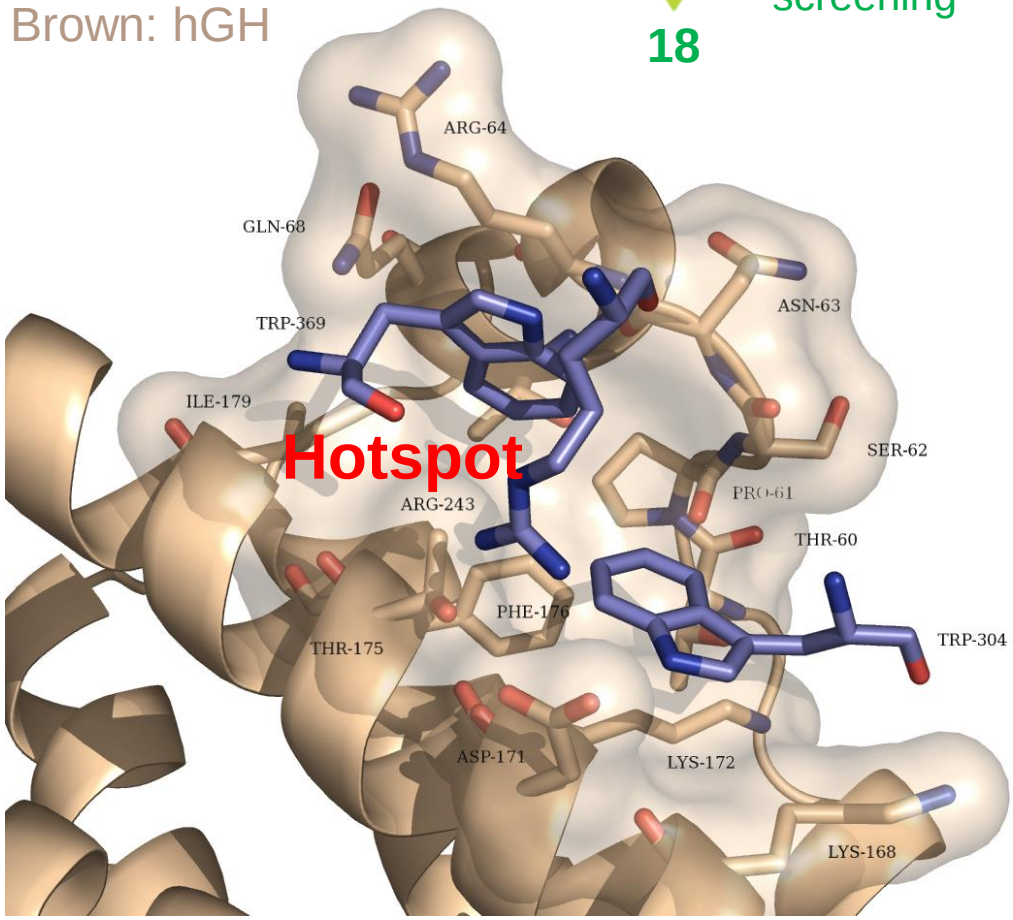
2715 BB's

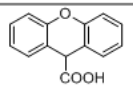
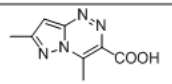
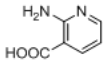
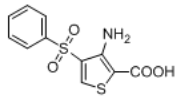
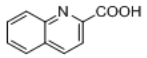
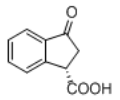
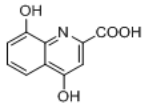
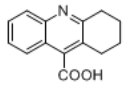
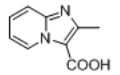
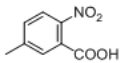
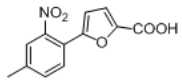
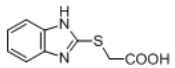
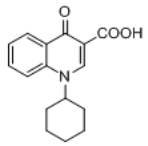
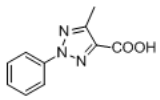
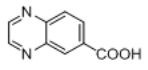
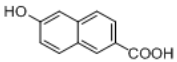
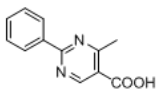
Virtual screening

18

Blue: receptor

Brown: hGH



R^3_n[a]	Structure	R^3_n	Structure
1		10	
2		11	
3		12	
4		13	
5		14	
6		15	
7		16	
8		17	
9		18	

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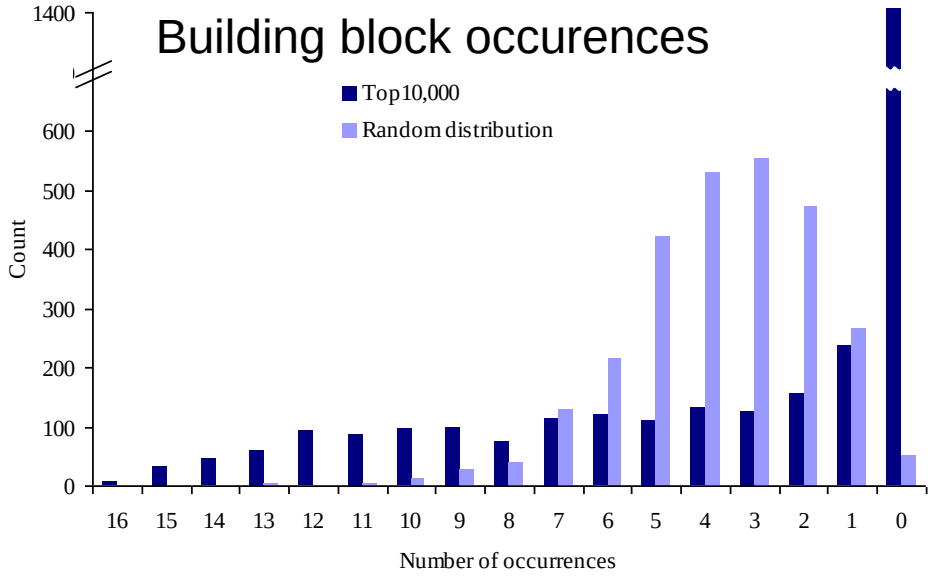
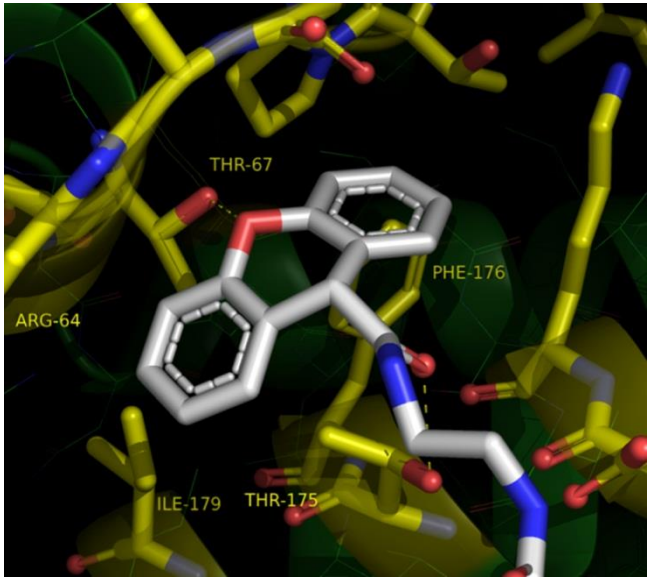
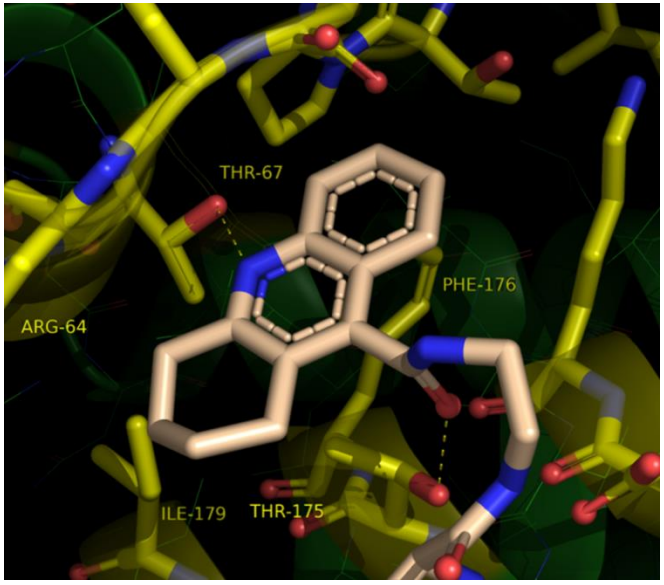


3-Residue virtual library 328515 ligands totaly: 30000 selected by prefiltering

This was further reduced to 2178 compounds redundancy elimination





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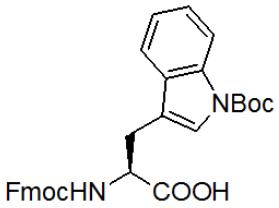
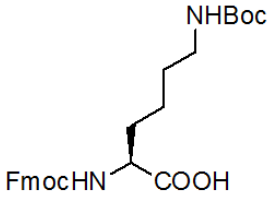
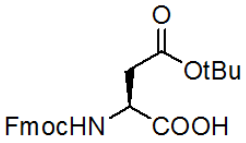
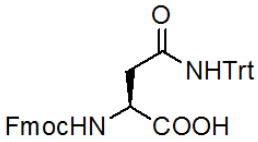
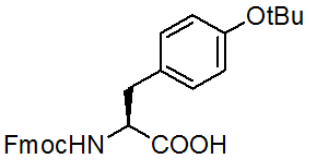
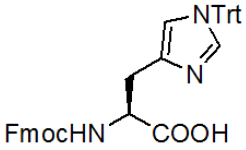
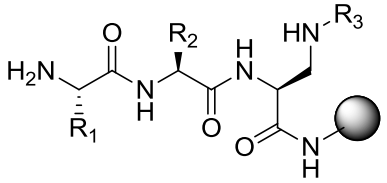
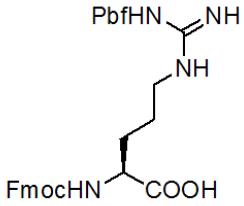
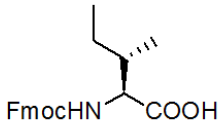
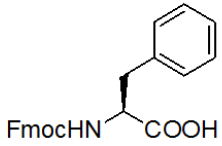
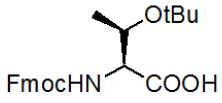
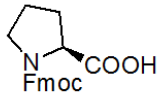
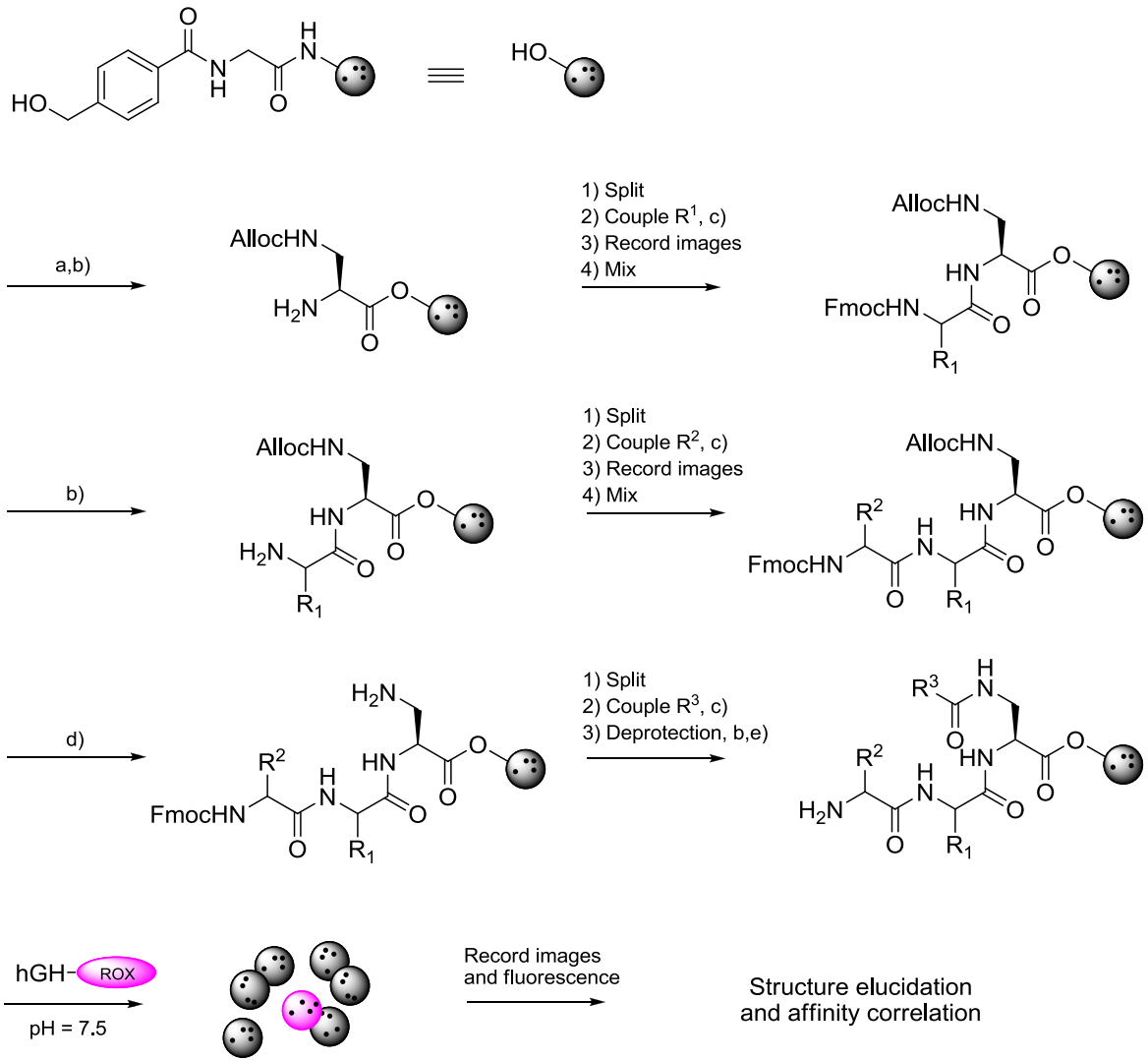
R^1_1 R^2_1	
R^1_2 R^2_2	
R^1_3 R^2_3	
R^1_4 R^2_4	
R^1_5 R^2_5	
R^1_6 R^2_6	



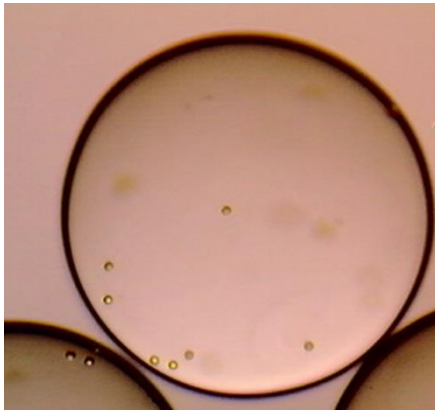
Selection of R1 R2:
Aromatic, basic, acidic, lipophilic, HB

R^1_7 R^2_7	
R^1_8 R^2_8	
R^1_9 R^2_9	
R^1_{10} R^2_{10}	
R^1_{11} R^2_{11}	

The "One Bead SOME Compounds" Solid Phase Assay



2178 compounds
on 50000 beads



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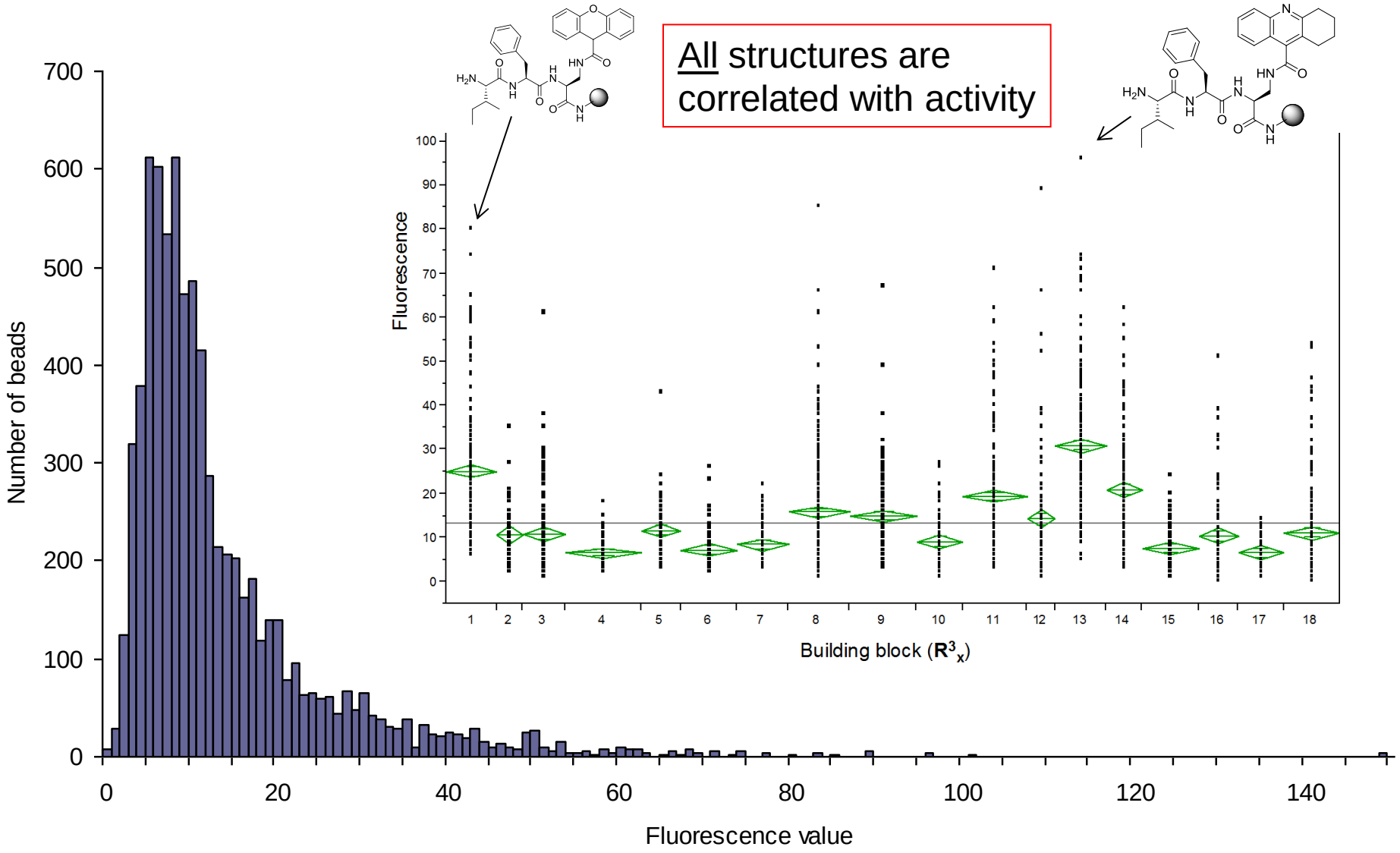
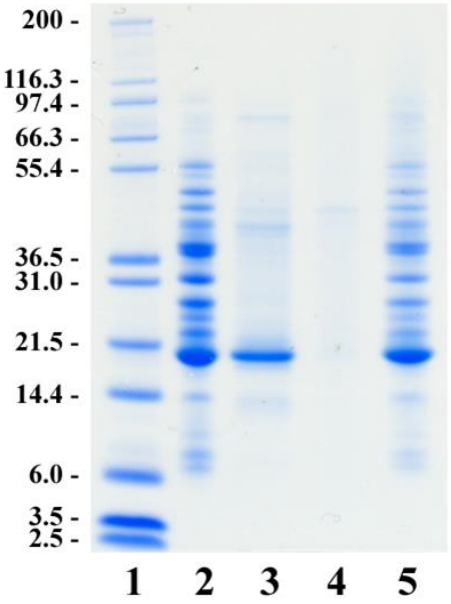
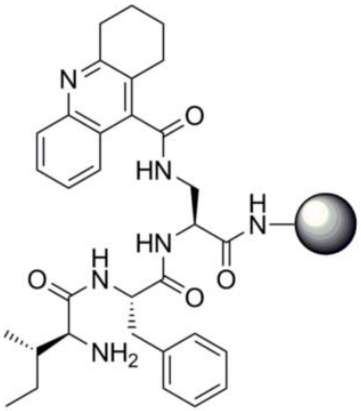
All structures are correlated with activity



Purification of hGH on affinity column





Affinity resin {9,8,13}