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Supporting information for article:

A complement to the modern crystallographer's toolbox: caged gadolinium complexes with versatile binding modes

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Table S1 Obtention of derivative crystals

If not mentioned otherwise, complex concentration for soaking or co-crystallisation was 100 mM and soaking time 1 hour. Glucose isomerase could not be co-crystallised with Gd-DTPA or Gd-DO3A, both complexes leading to precipitation of the protein, preventing crystal formation. Gd-DOTA-BOM had a solubilizing effect on lysozyme and thaumatin, thus co-crystals could only be obtained with a complex concentration of 50 mM. For the Gd-DOTA-BOM lysozyme derivative, precipitant concentration needed to be increased in order to obtain crystals, which were few in number and large in size. Gd-DO3A derivative crystals of urate oxidase could only be obtained with a maximum complex concentration of 50 mM. Lysozyme derivative crystals could only be obtained by co-crystallisation. Soaking trials led crystal cracking.

	Urate oxidase	Urate oxidase	Glucose isomerase	Thaumatin	Thaumatin
Gd-DO3A	co-crystallisation 50 mM	soaking 45 min	soaking 60 min	co-crystallisation	
Gd-HPDO3A	co-crystallisation		co-crystallisation	co-crystallisation	
Gd-DTPA-BMA	co-crystallisation	soaking, 300 mM 45 min	co-crystallisation	co-crystallisation	soaking 300 mM 45 min
Gd-DOTA	co-crystallisation		co-crystallisation	co-crystallisation	soaking 300 mM 45 min
Gd-DOTMA	co-crystallisation	soaking, 300 mM 45 min	co-crystallisation	co-crystallisation	
Gd-DTPA	co-crystallisation		soaking 60 min	co-crystallisation	soaking 300 mM 45 min
Gd-DOTA-BOM	co-crystallisation		co-crystallisation	co-crystallisation 50 mM	soaking 300 mM 45 min

	Lysozyme	Lysozyme	YGGV	YeaZ
Gd-DO3A	co-crystallisation	co-crystallisation 300 mM	soaking 50 -100 mM	soaking 6 hrs
Gd-HPDO3A	co-crystallisation	co-crystallisation 300 mM	soaking 50 -100 mM	
Gd-DTPA-BMA	co-crystallisation		soaking 50 -100 mM	soaking 6 hrs
Gd-DOTA	co-crystallisation	co-crystallisation 300 mM	soaking 50 -100 mM	
Gd-DOTMA	co-crystallisation		soaking 50 -100 mM	soaking 6 hrs
Gd-DTPA	co-crystallisation		soaking 50 -100 mM	
Gd-DOTA-BOM	co-crystallisation 50 mM		soaking 50 -100 mM	

Table S2 Data-collection parameters and processing statistics for derivative crystals obtained with the seven Gd complexes.

Values in parentheses refer to the highest resolution shell.

Lysozyme	Gd-DOTA-BOM 300 mM	Gd-DO3A 300 mM	Gd-HPDO3A 300 mM	Gd-DOTA 300 mM	
Space group	$P4_32_12$	$P4_32_12$	$P4_32_12$	$P4_32_12$	
Unit-cell parameters (Å)	a = b = 77.8, c = 37.4	a = b = 77.3, c = 37.3	a = b = 77.1, c = 38.3	a = b = 77.2, c = 38.4	
λ (Å)	0.98	0.98	0.98	0.98	1.54
X-ray source	BM30A	BM30A	BM30A	BM30A	CuKα
Resolution range (Å)	1.53 (1.53-1.62)	1.54 (1.54-1.62)	1.53 (1.53-1.62)	1.54 (1.54-1.62)	1.64 (1.64-1.73)
I/σ	5.4 (1.1)	6.6 (2.1)	6.3 (5.9)	8.3 (3.6)	6.7 (3.5)
<I/σ>	25.8 (3.0)	31.0 (3.8)	37.7 (8.0)	50.9 (7.1)	40.1 (12.3)
Multiplicity	12.4 (6.4)	12.5 (6.8)	12.7 (6.4)	12.7 (6.6)	11.9 (9.2)
R_{fac}	0.068 (0.387)	0.061 (0.26)	0.057 (0.079)	0.040 (0.15)	0.055 (0.086)
R_{ano}	0.034 (0.138)	0.039 (0.13)	0.050 (0.076)	0.035 (0.098)	0.062 (0.096)
Oscillation range	180°	180°	180°	180°	180°

Urate oxidase	Gd-DOTMA 300 mM		Gd-DTPA-BMA 300 mM		Gd-DO3A 100 mM
Space group	I222		I222		I222
Unit-cell parameters (Å)	a = 79.1, b = 95.0, c = 104.2		a = 79.6, b = 95.0, c = 104.3		a = 80.4, b = 95.5, c = 104.6
λ (Å)	0.98	1.711	0.92	1.711	0.98
X-ray source	ID29	ID29	BM30A	BM30A	BM30A
Resolution range (Å)	1.34 (1.34-1.41)	2.16 (2.16-2.28)	1.45 (1.45-1.53)	2.70 (2.70-2.84)	1.45 (1.45-1.53)
I/σ	9.1 (2.2)	7.4 (1.8)	9.1 (4.2)	13.9 (11.6)	10.7 (2.9)
<I/σ>	24.3 (2.8)	21.8 (2.2)	30.6 (5.9)	35.0 (15.0)	31.2 (2.1)
Multiplicity	6.6 (4.0)	6.5 (4.0)	6.8 (3.8)	6.2 (3.6)	6.2 (4.2)
R_{fac}	0.051 (0.32)	0.090 (0.37)	0.040 (0.13)	0.037 (0.051)	0.039 (0.19)
R_{ano}	0.037 (0.23)	0.098 (0.24)	0.044 (0.097)	0.110 (0.148)	0.023 (0.13)
Oscillation range	180°	180°	180°	180°	180° by 1° steps

Thaumatin	Gd-DOTA 300 mM		Gd-DOTA-BOM 300 mM	
Space group	$P4_12_12$		$P4_12_12$	
Unit-cell parameters (Å)	a = b = 58.0, c = 150.8		a = b = 57.8, c = 150.5	
λ (Å)	0.92	1.711	0.92	1.711
X-ray source	BM30A	BM30A	BM30A	BM30A
Resolution range (Å)	1.45 (1.45-1.53)	2.69 (2.69-2.83)	1.45 (1.45-1.53)	2.69 (2.69-2.84)
I/σ	6.7 (1.8)	17.4 (10.0)	6.8 (2.6)	8.1 (6.3)
<I/σ>	33.4 (4.0)	37.3 (18.6)	32.9 (3.8)	32.3 (9.1)
Multiplicity	12.6 (6.7)	5.4 (3.2)	13.0 (7.0)	12.2 (6.8)
R_{fac}	0.056 (0.24)	0.030 (0.056)	0.053 (0.24)	0.066 (0.096)
R_{ano}	0.019 (0.079)	0.054 (0.066)	0.029 (0.11)	0.096 (0.013)
Oscillation range	180°	80°	180°	180°

Thaumatin	Gd-DTPA-BMA 300 mM		Gd-DTPA 300 mM		Gd-HPDO3A 100 mM
Space group	$P4_12_12$		$P4_12_12$		$P4_12_12$
Unit-cell parameters (Å)	a = b = 57.9, c = 150.4		a = b = 57.9, c = 150.1		a = b = 57.9, c = 150.5
λ (Å)	0.92	1.711	0.92	1.711	1.54
X-ray source	BM30A	BM30A	BM30A	BM30A	CuKα
Resolution range (Å)	1.45 (1.45-1.53)	2.66 (2.66-2.81)	1.45 (1.45-1.53)	2.67 (2.67-2.82)	1.55 (1.55-1.63)
I/σ	9.0 (3.5)	11.4 (9.6)	10.3 (5.9)	14.8 (11.3)	9.2 (8.1)
<I/σ>	44.9 (7.8)	45.2 (18.0)	49.7 (11.0)	57.9 (21.3)	57.9 (18.6)
Multiplicity	13.0 (7.2)	12.0 (6.3)	12.8 (6.9)	11.6 (6.0)	16.3 (13.9)
R_{fac}	0.040 (0.15)	0.043 (0.063)	0.037 (0.097)	0.032 (0.052)	0.039 (0.092)
R_{ano}	0.021 (0.053)	0.058 (0.072)	0.025 (0.042)	0.049 (0.062)	0.024 (0.037)
Oscillation range	180°	180°	180°	180°	217°

Glucose isomerase	Gd-DO3A 100 mM		Gd-DTPA 100 mM	
Space group	I222		I222	
Unit-cell parameters (Å)	a = 93.0, b = 98.3, c = 102.6		a = 93.0, b = 98.2, c = 102.6	
λ (Å)	0.92	1.711	0.92	1.711
X-ray source	BM30A	BM30A	BM30A	BM30A
Resolution range (Å)	1.44 (1.44-1.52)	2.69 (2.69-2.83)	1.45 (1.45-1.53)	2.68 (2.68-2.83)
I/σ	10.3 (1.4)	17.1 (6.5)	8.3 (1.5)	15.4 (8.1)
<I/σ>	35.6 (4.9)	39.2 (8.3)	33.4 (3.8)	41.8 (10.8)
Multiplicity	6.8 (3.5)	6.5 (3.5)	6.9 (4.2)	6.6 (4.1)
R_{fac}	0.036 (0.29)	0.034 (0.088)	0.043 (0.29)	0.034 (0.072)
R_{ano}	0.027 (0.13)	0.082 (0.102)	0.024 (0.112)	0.039 (0.060)
Oscillation range	180°	180°	180°	180°

Glucose isomerase	Gd-DOTA-BOM 100 mM		Gd-DOTMA 100 mM		Gd-DTPA-BMA 100 mM
Space group	I222		I222		I222
Unit-cell parameters (Å)	a = 92.7, b = 98.3, c = 102.3		a = 92.7, b = 98.0 ,c = 102.6		a = 92.7, b = 97.9, c = 102.6
λ (Å)	0.92	1.711	0.92	1.711	1.54
X-ray source	BM30A	BM30A	BM30A	BM30A	CuKα
Resolution range (Å)	1.43 (1.43-1.51)	2.68 (2.68-2.82)	1.44 (1.44-1.51)	2.68 (2.68-2.82	1.75 (1.75-1.84)
I/σ	13.0 (1.1)	21.0 (8.0)	9.2 (1.4)	12.2 (5.6)	7.8 (4.0)
<I/σ>	30.1 (5.4)	44.3 (10.2)	23.8 (3.7)	25.2 (6.0)	20.1 (7.2)
Multiplicity	4.7 (2.6)	6.6 (3.7)	6.8 (3.5)	5.3 (3.0)	7.3 (6.1)
R_{fac}	0.031 (0.33)	0.030 (0.078)	0.050 (0.31)	0.047 (0.11)	0.059 (0.16)
R_{ano}	0.024 (0.20)	0.033 (0.056)	0.023 (0.18)	0.038 (0.092)	0.022 (0.082)
Oscillation range	180°	180°	180°	145°	204°

YGGV	Gd-DO3A 100 mM		
Space group	$P4_32_12$		
Unit-cell parameters (Å)	a = b = 78.7, c = 77.2		
λ (Å)	peak, 1.711	infl1, 1.712	infl2, 1.711
X-ray source	BM30A	BM30A	BM30A
Resolution range (Å)	2.68 (2.68-2.82)	2.68 (2.68-2.83)	2.68 (2.68-2.82)
I/σ	8.9 (1.4)	10.4 (1.6)	10.4 (1.6)
<I/σ>	26.2 (12.6)	19.5 (10.8)	19.3 (10.6)
Multiplicity	12.2 (5.3)	6.2 (3.4)	6.2 (3.3)
R_{fac}	0.066 (0.47)	0.050 (0.31)	0.051 (0.29)
R_{ano}	0.106 (0.24)	0.063 (0.28)	0.065 (0.23)
Oscillation range	180°	90°	90°

YGGV	Gd-DOTA-BOM 100 mM			Gd-DOTMA100 mM	
Space group	$P4_32_12$			$P4_32_12$	
Unit-cell parameters (Å)	a = b = 79.7, c = 78.23			a = b = 79.7, c = 78.3	
λ (Å)	peak 1.712	infl1 1.712	infl2 1.711	peak 1.712	infl1 1.712
X-ray source	BM30A	BM30A	BM30A	BM30A	BM30A
Resolution range (Å)	2.68 (2.68-2.82)	2.68 (2.68-2.82)	2.68 (2.68-2.82)	2.69 (2.69-2.84)	2.70 (2.70-2.84)
I/σ	7.7 (1.7)	11.2 (3.4)	11.1 (3.2)	8.1 (5.5)	9.7 (7.1)
<I/σ>	21.1 (10.2)	28.5 (13.6)	27.2 (13.5)	23.6 (8.2)	23.1 (9.5)
Multiplicity	8.1 (3.7)	6.2 (3.2)	6.2 (3.2)	8.3 (4.7)	5.8 (3.3)
R_{fac}	0.061 (0.24)	0.037 (0.14)	0.039 (0.15)	0.061 (0.12)	0.053 (0.086)
R_{ano}	0.073 (0.16)	0.040 (0.11)	0.047 (0.13)	0.079 (0.12)	0.042 (0.062)
Oscillation range	120°	90°	90°	120°	82.5°

YEAZ	Gd-DOTMA 100 mM		Gd-DO3A 100 mM
Space group	$P2_12_12_1$		$P2_12_12_1$
Unit-cell parameters (Å)	a = 76.3, b = 97.6, c = 141.9		a = 76.1, b = 97.2, c = 142.1
λ (Å)	1.711	0.98	1.71
X-ray source	BM30A	BM30A	BM30A
Resolution range (Å)	2.70	2.28	31.782 - 3.35 (3.27-3.13)
I/σ	13.6 (6.9)	9.3 (1.7)	9.7 (2.2)
Multiplicity	4.7 (1.3)	5.0 (2.6)	3.4 (3.4)
R_{fac}	0.034 (0.096)	0.054 (0.23)	0.17 (0.58)
R_{ano}	0.043 (0.094)	0.028 (0.13)	0.16 (0.34)

Table S3 Phasing statistics for derivative crystals obtained with the seven Gd complexes.

FOM, FOMshasol, FOMdm, figures of merit for initial phases, for phases after density modification by SOLOMON and by DM. FOM (2.7), FOM (2.2), figure of merit for general reflections, for resolution up to 2.7 Å and 2.2 Å, respectively (correspond to resolution limits on Beamlines BM30A and ID29 respectively for the peak wavelength). Qsha and Bsha, occupancy and B-factor of Gd atoms refined by SHARP. R_{free}, direct space residual factor calculated by DM based on protein regions that are not included in the density modification.

$$R_{free} = \frac{\sum \rho_{obs} - \rho_{calc}}{\sum \rho_{obs} + \rho_{calc}}.$$

peak, infl1, infl2, wavelengths at absorption edge with *f''* maximum, *f'* minimum and a third, shorter wavelength respectively. CuKα:data collected on in-house rotating Cu-anode X-ray generator.

Lysozyme	Gd-DOTA-BOM	Gd-DO3A	Gd-HPDO3A	Gd-DOTA
Method	SAD	SAD	SAD	MAD
_ [Å]	0.98	0.98	0.98	0.98, 1.54
X-ray source	BM30A	BM30A	BM30A	BM30A, CuKα
Resolution [Å]	55.1-1.53	54.6-1.54	54.5- 1.53	54.6- 1.54
Nb sites	1	4	2	2
Qsha	0.78	0.75, 1.02, 0.35,	1.06, 0.85	0.82, 0.52
Bsha [$Å^2$]	27	27, 45, 23, 15	15, 12	16, 14
FOM	0.25	0.31	0.58	0.58
FOM (2.7)				0.70
FOMshasol	0.75	0.75	0.91	0.88
FOMdm	0.71	0.70	0.91	0.77
R_{free}	0.22	0.18	0.14	0.17

Urate oxidase	Gd-DOTMA 200 µM	Gd-DTPA-BMA 200 µM	Gd-DO3A 100 µM
Method	MAD	MAD	SAD
_ [Å]	0.98, 1.71	0.92, 1.71	0.98
X-ray source	ID29	BM30A	BM30A
Resolution [Å]	70.2 - 1.34	70.2 - 1.45	70.5 - 1.45
Nb sites	1	3	3
Qsha	1.08, 0.31, 0.22	0.87, 0.66, 0.82	0.03, 0.02, 0.17
Bsha [Å^2]	25, 30, 30	14, 15, 14	30, 30, 30
FOM	0.23	0.53	0.071
FOM (2.7)	0.57	0.67	
FOMshasol	0.86	0.92	0.77
FOMdm	0.87	0.84	0.67
R_{free}	0.21	0.17	0.20

Thaumatin	Gd-DOTA 200 µM	Gd-DOTA-BOM 200 µM	Gd-DTPA-BMA 200 µM	Gd-DTPA 200 µM	Gd-HPDO3A 100 µM
Method	MAD	MAD	MAD	MAD	SAD
_ [Å]	0.92, 1.71	0.92, 1.71	0.92, 1.71	0.92, 1.71	1.54
X-ray source	BM30A	BM30A	BM30A	BM30A	CuKα
Resolution [Å]	54.2 - 1.45	53.9 - 1.45	54.1 - 1.45	54.0- 1.45	54.2 - 1.55
Nb sites	1	2	1	1	3
Qsha	0.61	0.93, 0.48	0.70	0.48	0.15, 0.29, 0.14
Bsha [Å^2]	29	22, 17	28	16	15, 30, 23
FOM	0.26	0.42	0.35	0.32	0.21
FOM (2.7)	0.49	0.71	0.57	0.51	
FOMshasol	0.88	0.91	0.90	0.91	0.88
FOMdm	0.92	0.88	0.89	0.89	0.82
R_{free}	0.108	0.090	0.128	0.096	0.113

Glucose isomerase	Gd-DO3A 100 µM	Gd-DTPA 100 µM	Gd-DOTA-BOM 100 µM	Gd-DOTMA 100 µM	Gd-DTPA-BMA 100 µM
Method	MAD	MAD	MAD	SAD	SAD
_ [Å]	0.92, 1.71	0.92, 1.71	0.92, 1.71	1.54	1.54
X-ray source	BM30A	BM30A	BM30A	CuKα	CuKα
Resolution [Å]	70.7 - 1.44	70.7 - 1.45	70.7 - 1.43	70.7 - 1.76	70.7 - 1.76
Nb sites	3	2	2	1	3
Qsha	0.78, 0.75, 0.46	0.47, 0.12	0.21, 0.22	0.40	0.13, 0.08, 0.25
Bsha [Å^2]	anisotropic	22.05, 20	16, 13	28	43, 4, 25
FOM	0.36	0.22	0.15	0.12	0.19
FOM (2.7)	0.57	0.44	0.31		
FOMshasol	0.86	0.88	0.82	0.79	0.85
FOMdm	0.84	0.88	0.85	0.75	0.78
R_{free}	0.174	0.123	0.199	0.147	0.191

YggV	Gd-DO3A 100 mM	Gd-DOTA-BOM 100 mM	Gd-DOTMA 100 mM
Method	MAD	MAD	MAD
_ [Å]	peak, infl1, infl2	peak, infl1, infl2	peak, infl1
X-ray source	BM30A	BM30A	BM30A
Resolution [Å]	55.9 - 2.68	55.9 - 2.68	55.9 - 2.70
Nb sites	4	4	3
Qsha	0.71, 0.37, 0.13, 0.18	0.28, 0.26, 0.21, 0.21	0.20, 0.13, 0.13
Bsha [Å^2]	64, 92, 33, 45	-------	33, 66, 63
FOM	0.67	0.48	0.68
FOMshasol	0.83	0.79	0.90
FOMdm	0.77	0.65	0.85
R_{free}	0.111	0.161	0.100

YeaZ	Gd-DOTMA 100 mM
Method	MAD
_ [Å]	peak, 0.98
X-ray source	BM30A
Resolution [Å]	80.3-2.28
Nb sites	8
Qsha	0.55, 0.35, 0.31, 0.09, 0.22, 0.22, 0.22, 0.22
Bsha [Å^2]	20, 62, 102, 1, 49, 49, 49, 49
FOM	0.44
FOMshasol	0.82
FOMdm	0.79
R_{free}	0.123

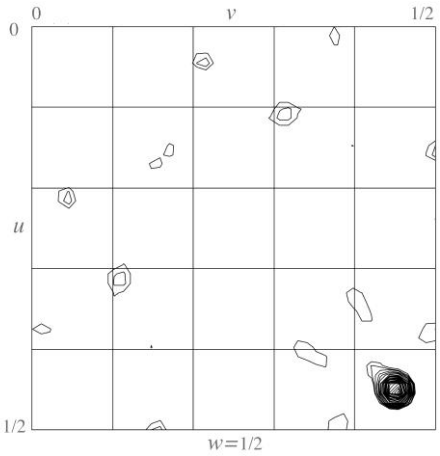

Figure S1 Anomalous Patterson map for the YggV Gd-DOTMA derivative. Harker section w=1/2.