Supplementary Information (SI) for Nanoscale.

ELECTRONIC SUPPLEMENTARY INFORMATION

Endovascular administration and magnetic retention of nanocapsules for improved brain delivery in large cerebral vascular models.

Grayston et al. Nanoscale.

SUPPLEMENTARY METHODS

Nanocapsules synthesis

Briefly, SPIONs were embedded in the PLGA shell by adding oleic acid-coated SPIONs, with an average diameter of 9 nm, in the PLGA organic phase during the first emulsification following previously described methods.[9,10] Modified PLGA-Cy5 was also added in the organic phase in addition to the commercial PLGA (Resomer® RG502H, acid terminated, MW 7000-17000, Sigma-Aldrich, CAS: 26780-50-7). As the first aqueous phase, 50 µL of aqueous solution at physiological pH (EBM2, Lonza), were added during the double emulsion solvent evaporation method for a single NCs batch. The obtained NCs suspension in 2 mg/mL trehalose aqueous solution was lyophilized and stored at -80 ºC until each experimental use. The NC were freshly dispersed at the desired concentration before use, vortexed for 1 min and sonicated in an ultrasound bath for 3 min at 83.3 W/L and 48 kHz.

Dynamic light scattering

The hydrodynamic diameter of the nanocapsules was measured by dynamic light scattering (DLS) with a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). Samples were diluted to a final concentration of 0.1 mg/mL in ultrapure water and measured in triplicate at 25 ºC.

Transmission electron microscopy

The morphology of the magnetic nanocapsules was studied by transmission electron microscopy (TEM) using a JEOL JEM-1210 microscope. For sample preparation, a drop of a diluted suspension of magnetic PLGA-NC in ultrapure water (0.5 mg/mL) was placed onto a copper grid and allowed to settle for 5 min. The excess liquid was gently removed with filter paper, and the procedure was repeated 2 more times before imaging.

Magnetic characterization

The magnetic properties of the PLGA-NC were measured in a superconducting quantum interference device (SQUID) (MPMS5XL magnetometer, Quantum Design, CA, United States). The nanocapsules in powder form (5 mg) were compacted into a spherical shape using PTFE tape and its hysteresis loops were measured at 10 and 300 K under a maximum applied magnetic field of ±60 kOe.

Animals

Pigs (Large White x Landrace cross) were purchased from the animal centre A. M. Animalia Bianya S.L. (Girona, Spain), entered in the register of breeding centres, suppliers and users of laboratory animals with the number G9900009 (GLP nº BPL808CAT). The animals were previously acclimatized to our facilities and housed in conventional pens for 7-10 days, with *ad libitum* water and fed 2 times a day with conventional pig's diet. The animals were housed with other pigs, and the pen's temperature was maintained at 21±2ºC. The relative humidity ranged from 40-60%, air renovation consisted of 10-15 air changes per hour, and the photoperiod was 12:12 h day/night. All animals were subjected to a clinical examination by the veterinary team, and underwent a fasting of 12 h prior to surgery, maintaining free access to water with diluted sucrose. The animals were kept under general anesthesia for the entire duration of the intervention and subsequent experiment to administer intra-arterial or intravenous NC as described in the main text.

3D printing

Briefly, the neurovascular model manufacturing comprised the following steps: (i) medical image segmentation to generate the 3D geometry of the vascular anatomy, (ii) mesh modelling to prepare a printable anatomical model, (iii) 3D printing configuration, and (iv) post-printing processing. DICOM data from intravenous contrast computed tomography scans from anonymous patients were imported and loaded in the 3DSlicer software to obtain the preliminary 3D geometry of the model, through opacity threshold segmentation. Then, using the Autodesk Meshmixer software, the 3D geometry was simplified into anatomically relevant vessels for mechanical thrombectomy, that is to say, carotid arteries (CCAs, ECAs, ICAs), MCAs, anterior carotid arteries (ACAs), anterior

communicating artery (ACoA), vertebral arteries (VAs), basilar artery (BA), posterior cerebral arteries (PCAs), and posterior communicating arteries (PCoAs). The proximal and distal segments were adapted to create connection points of the model to the flow loop system, and 1 mm thickness was added to the vessel walls to provide consistency. Finally, mesh errors were corrected, and the virtual model was exported from Autodesk Meshmixer to PreForm to configure the printing material, resolution, optimal geometry orientation, and support structure for 3D printing. The model was printed with commercially available photopolymer clear resin (Clear, FormLabs, Boston, MA, USA) at a resolution of 100 μm with a Form 3 Printer (FormLabs). For post-printing processing, the support structures were removed from the model, which was then immersed in isopropyl alcohol and exposed to 305 nm UV light at 60ºC for 20 min. A temporal bone was also 3D-printed using the same methodology, using the scaffold material Durable resin (FormLabs) with a final average thickness of 4.46 mm (3.5-9.86 mm).

Fluorescence Molecular Imaging

For *in vitro* FMI, a series of concentrations ranging from 0.1 mg/mL to 1.5 mg/mL of Cy5-labelled NC in 100 μL of saline were prepared for each batch as well as a background control well (0 mg/mL) in a 96-well plate and imaged using an IVIS® Lumina LT Series III imaging system (Perkin Elmer, (λex/λem 640/732 nm, respectively); Waltham, MA). To evaluate the specific magnetic retention in the 3D-printed vascular model, circular ROIs were manually drawn on both MCAs and corrected by the TRE from the corresponding ROI in an empty vascular model. For *ex vivo* FMI, TRE was quantified on ROIs manually drawn for the whole pig brains or the corresponding ipsilateral and contralateral hemispheres.

SUPPLEMENTARY FIGURES

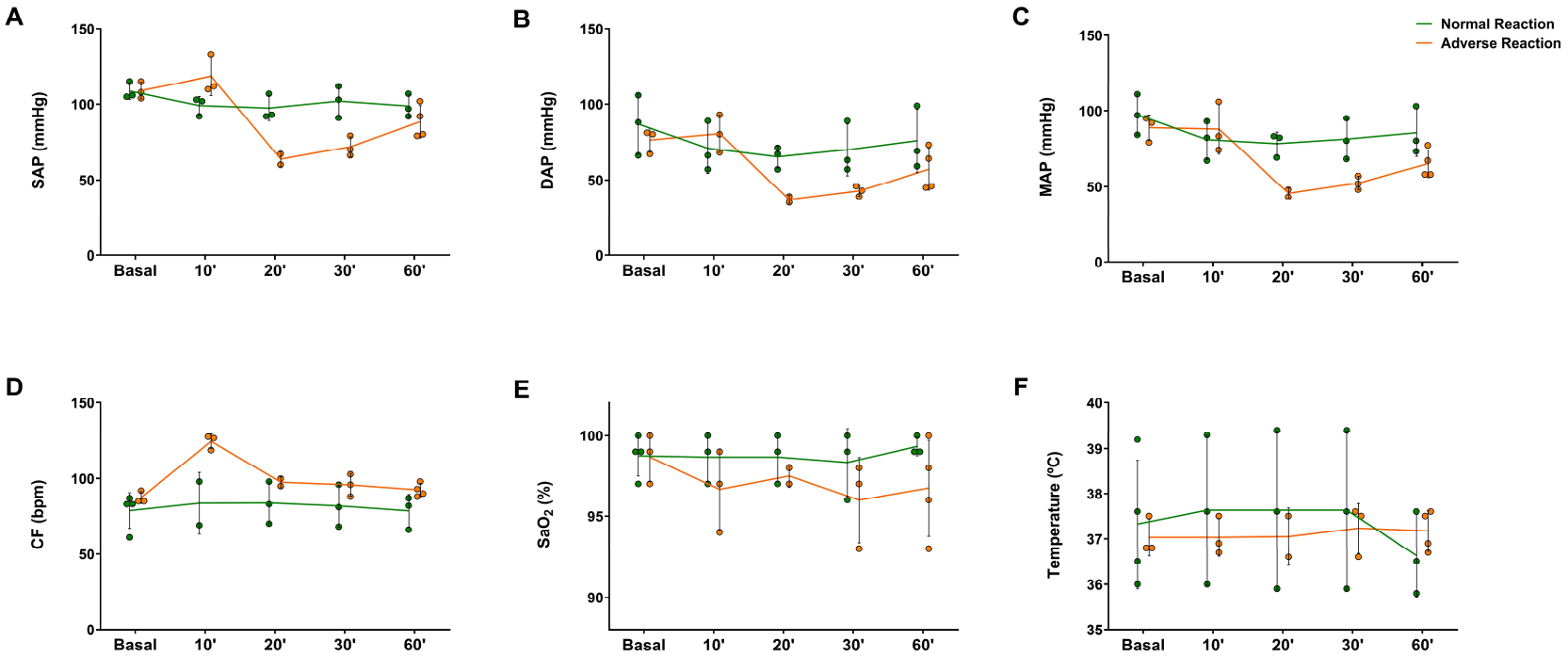

Figure S1. Physiological parameters measured during nanocapsule infusion. Timeline bar-graphs showing Systolic Arterial Pressure (SAP), Diastolyc Arterial Pressure (DAP), Mean Arterial Pressure (MAP), Cardiac Frequency (CF), arterial oxygen saturation (SaO_2) and body temperature at different time points during the 60 minutes nanocapsule infusion in subjects suffering adverse reaction or not. Data represent mean±SD or median (IQR) and individual values are represented. Only individuals with the complete temporal profile (n=5/7 are represented).

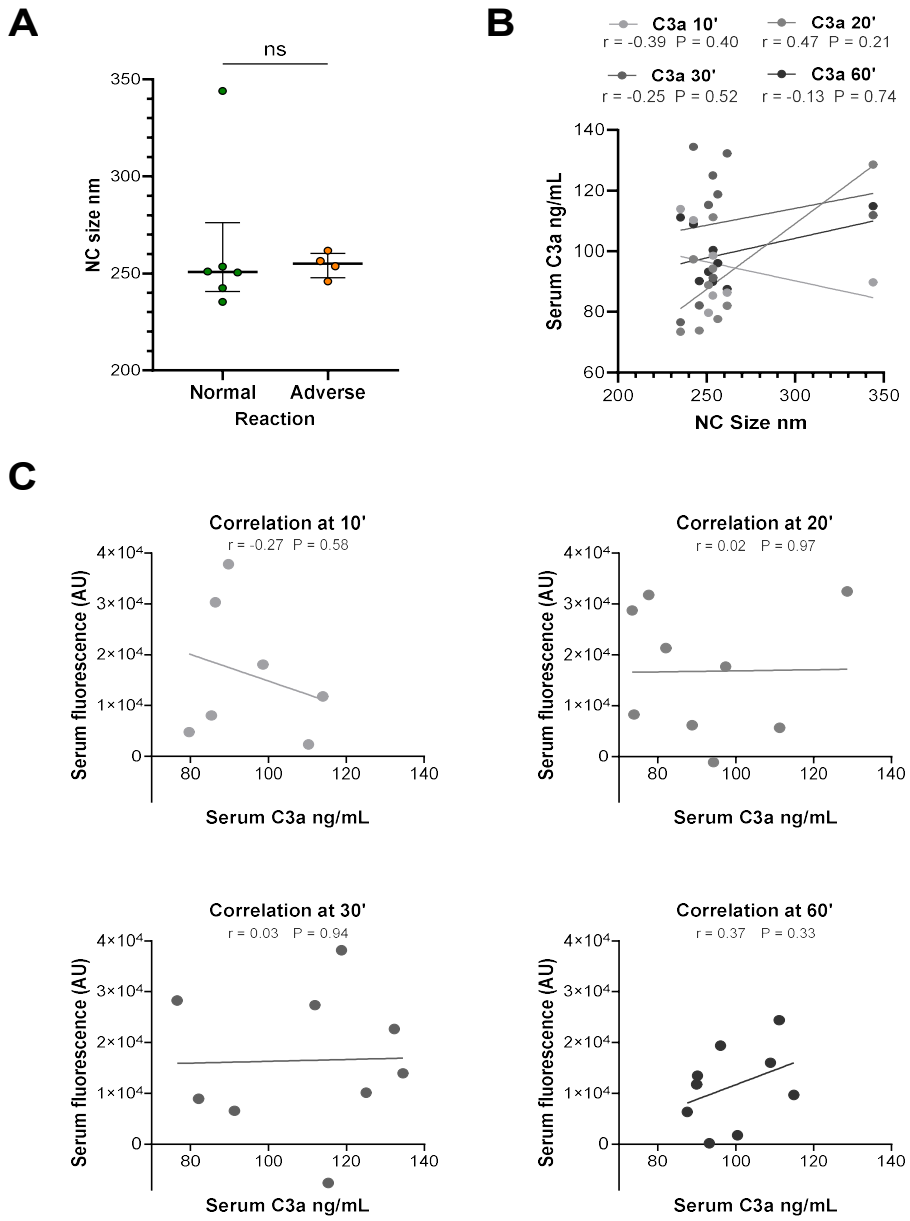

Figure S2. Nanocapsule association with C3a levels and adverse effects during infusion.

A) graph showing that NC size was similar in animals suffering normal or adverse reactions post NC infusion. Data is represented as median (IQR). **B)** NC size correlation with serum complement fragment C3a levels at different timepoints during NC infusion. **C)** Serum C3a levels correlation with Cy5+ NC fluorescence at different timepoints during NC infusion. No significance was observed.

SUPPLEMENTARY DATA SUPPORTING THE EXPERIMENTAL ANALYSIS

Nanocapsule Size by batch.

Batch ID	Size (nm)	Polydispersity index (PDI)	Route	Batch ID	Size (nm)	Polydispersity index (PDI)	Route
NC219	256.0	0.13	IA	ASNC051	260.0	0.12	IV
NC224	466.0	0.45	IA	ASNC052	253.0	0.15	IV
NC225	310.0	0.26	IA	ASNC053	249.0	0.14	IV
NC251	276.0	0.20	IA	ASNC103	253.5	0.16	IA
NC252	256.0	0.15	IA	ASNC104	245.7	0.18	IV
NC253	260.0	0.15	IA	ASNC105	259.2	0.19	IA
NC254	269.0	0.16	IA	ASNC106	246.1	0.15	IV
NC257	229.0	0.10	IA	ASNC110	237.3	0.19	IV
ASNC048	243.0	0.10	IV	ASNC112	233.4	0.20	IV
ASNC049	247.0	0.11	IV	ASNC124	244.0	0.12	IA
ASNC050	242.0	0.12	IV	ASNC128	257.0	0.12	IA

Nanocapsule Fluorescence Quantification by batch.

NC concentration	ROI TRE									
(mg/mL)	NC batches used for intraarterial administration									
	NC219	**NC251**	**NC252**	**NC253**	**NC254**	**NC257**	**ASNC103**	**ASNC105**	**ASNC124**	**ASNC128**
0	1.0705E+11	0	0	0	0	0	0	0	0	0
0.1	2.151E+12	2.01E+12	2.29E+12	2.47E+12	3.29E+12	2.41E+12	2.85E+12	1.59E+12	1.8E+12	2.23E+12
0.25	4.744E+12	4.6E+12	5.74E+12	6.92E+12	7.59E+12	5.28E+12	5.53E+12	3.36E+12	3.63E+12	4.32E+12
0.5	8.953E+12	8.45E+12	1E+13	1.23E+13	1.57E+13	1.13E+13	7.75E+12	6.79E+12	6.36E+12	7.91E+12
0.75	1.452E+13	1.4E+13	1.55E+13	1.88E+13	2.46E+13	1.73E+13	1.35E+13	1.11E+13	8.52E+12	1.13E+13
1	1.8325E+13	1.76E+13	2.14E+13	2.5E+13	3.16E+13	2.15E+13	1.71E+13	1.46E+13	1.08E+13	1.49E+13
1.5	2.5055E+13	2.43E+13	2.9E+13	3.3E+13	3.98E+13	3.23E+13	2.81E+13	2.38E+13	1.55E+13	2.02E+13
	ASNC048	**ASNC049**	**ASNC050**	**ASNC051**	**ASNC052**	**ASNC053**	**ASNC104**	**ASNC106**	**ASNC110**	**ASNC112**
0	0	0	0	0	0	0	0	0	0	0
0.1	2.06E+12	2.43E+12	1.55E+12	2.7E+12	2.91E+12	3.27E+12	2.34E+12	2.12E+12	2.43E+12	2.38E+12
0.25	6.29E+12	6.84E+12	4.94E+12	8.2E+12	8.59E+12	8.64E+12	5.2E+12	5.07E+12	6.13E+12	5.03E+12
0.5	6.66E+12	9.34E+12	6.94E+12	9.97E+12	9.42E+12	9.46E+12	9.66E+12	1.14E+13	8.6E+12	8.81E+12
0.75	1.21E+13	1.51E+13	8.43E+12	1.63E+13	1.69E+13	1.59E+13	1.53E+13	1.66E+13	1.39E+13	1.24E+13
1	1.6E+13	1.92E+13	1.63E+13	2.3E+13	2.15E+13	2.03E+13	1.9E+13	2.27E+13	1.69E+13	1.73E+13
1.5	2.45E+13	2.88E+13	2.74E+13	3.15E+13	3.06E+13	2.94E+13	3.09E+13	1.79E+13	2.16E+13	2.34E+13

Brain and Serum Fluorescence by Animal.

						IVIS						
	Administration Route		Effect		Mean NC Size	Total Radiant Efficiency (TRE)					Fold Change	
	Intravenous	Intraarterial	Normal	Adverse	nm	Whole Brain	IV - L	IV - R	IA - IL	IA - CL	IV L/R	IA IL/CL
PIG3		x	x		344.0	5.15E+13			3.40E+13	1.74E+13		1.96
PIG4		x		x	253.7	4.01E+14			2.90E+14	1.11E+14		2.63
PIG5		x		x	261.7	8.58E+14			7.92E+14	6.58E+13		12.04
PIG7	x		x		242.5	2.28E+13	1.17E+13	1.11E+13			1.06	
PIG8	x		x		253.5	2.68E+13	1.43E+13	1.25E+13			1.15	
PIG9	x		x		251.0	2.91E+13	1.44E+13	1.47E+13			0.98	
PIG10		x		x	256.4	2.85E+13			1.58E+13	1.27E+13		1.25
PIG11	x			x	245.9	2.79E+13	1.35E+13	1.44E+13			0.94	
PIG12	x		x		235.4	2.78E+13	1.46E+13	1.32E+13			1.11	
PIG14		x	x		250.5	7.86E+13			5.46E+13	2.41E+13		2.27

	Serum fluorescence λex 650 nm / λem 750 nm (AU)				Serum C3a levels (ng/mL)			
	Fluorescence 10'	Fluorescence 20'	Fluorescence 30'	Fluorescence 60'	C3a 10'	C3a 20'	C3a 30'	C3a 60'
PIG3	37817	32459	27344	9694	89.74	128.605	111.927	114.89
PIG4	18063	5629	6551	1763	98.589	111.252	91.249	100.438
PIG5	30333	21333	22667	6333	86.36	82.06	132.318	87.556
PIG7	2316	17644	13931	16007	110.305	97.386	134.483	108.941
PIG8	8033	-1163	10126	11775	85.367	94.298	125.04	89.942
PIG9	4771	6135	-7651	172	79.67	88.81	115.325	93.211
PIG10		31774	38136	19399		77.655	118.748	96.118
PIG11		8225	8900	13443		73.862	82.072	90.17
PIG12	11790	28737	28250	24387	113.96	73.411	76.542	111.162
PIG14	18528	34501	37011	41186				

Nanocapsule Quantification in Brain Samples.

Count NCs / mm2												
	IV-L			IV-R			IA-CL			IA-IL		
PIG3							0.05	0.28	2.34	0.25	0.26	0.71
PIG4							0.08	1.11	0.21	0.36	0.76	0.90
PIG5							0.68	0.29	0.04	0.98	0.72	0.84
PIG7	0.05	0.11	0.09	0.05	0.11	0.05						
PIG8	0.00	0.95	0.00	1.24	0.53	0.02						
PIG9	0.28	0.06	0.34	0.09	0.04	0.23						
PIG10							0.28	0.65	0.04	0.17	0.27	0.08
PIG11	0.07	0.08	0.05	0.18	0.34	0.03						
PIG12	0.12	0.03	0.12	0.73	0.00	0.02						
PIG14							0.08	0.15	0.00	0.03	0.28	0.18

Count NCs / Area - FC						
	IV- L/R			IA-IL/CL		
PIG3				5.01	0.90	0.30
PIG4				4.28	0.69	4.34
PIG5				1.44	2.52	20.33
PIG7	0.88	1.06	0.60			
PIG8		0.56				
PIG9	0.32	0.63	0.68			
PIG10				0.61	0.42	2.19
PIG11	2.60	4.22	0.52			
PIG12	6.10	0.00	0.18			
PIG14				0.35	1.86	

Fluorescence Quantification in the 3D-vascualr Model

Experiment #	Total Radiant Efficiency in MCA ROI					
	No magnet			Magnet		
	CL	IL	IL/CL	CL	IL	IL/CL
3122020				3.18E+13	1.29E+14	4.05
15122021				3.24E+13	1.53E+14	4.71
16122021				1.31E+14	3.82E+14	2.91
6022024				1.3E+14	1.95E+14	1.5
5022024	1.02E+14	1.11E+14	1.09			
6022024	1.44E+14	2.06E+14	1.43			
7022024	1.24E+14	1.88E+14	1.51			
6032024				1.86E+14	3.11E+14	1.67
6032024	1.18E+14	1.87E+14	1.59			

Vital Signs

SAP (mmHg)	Infusion time	Pig 3	Pig 7	Pig 8	Pig 9	**Normal Reaction (mean)**		Pig 4*	Pig 5*	Pig 10*	Pig 11*	**Adverse Reaction (mean)**
	Basal		115	106	105	**109**		104	115	108		**109**
	10 min	92		103	102	**99**		112	133		110	**118**
	20 min	92		93	107	**97**		60	67			**64**
	30 min	91		112	103	**102**		79	66		70	**72**
	Final		107	92	97	**99**		80	102	92	79	**88**

DAP (mmHg)	Infusion time	Pig 3	Pig 7	Pig 8	Pig 9	**Normal Reaction (mean)**		Pig 4*	Pig 5*	Pig 10*	Pig 11*	**Adverse Reaction (mean)**
	Basal		106	88	66	**87**		67	80	81		**76**
	10 min	57		89	66	**71**		80	93		68	**80**
	20 min	57		71	67	**65**		35	39			**37**
	30 min	57		89	63	**70**		46	39		43	**43**
	Final		99	69	59	**76**		46	64	73	45	**57**
MAP (mmHg)	Infusion time	Pig 3	Pig 7	Pig 8	Pig 9	**Normal Reaction (mean)**		Pig 4*	Pig 5*	Pig 10*	Pig 11*	**Adverse Reaction (mean)**
	Basal		111	97	84	**97**		79	92	95		**89**
	10 min	67		93	82	**81**		74	106		83	**88**
	20 min	69		82	83	**78**		43	48			**46**
	30 min	68		95	80	**81**		57	48		52	**52**
	Final		103	80	73	**85**		58	77	67	58	**65**
CF (bpm)	Infusion time	Pig 3	Pig 7	Pig 8	Pig 9	**Normal Reaction (mean)**		Pig 4*	Pig 5*	Pig 10*	Pig 11*	**Adverse Reaction (mean)**
	Basal	87	83	83	61	**79**		85	92	85		**87**
	10 min	98			69	**84**		119	127		128	**125**
	20 min	98		83	70	**84**		100	95			**98**
	30 min	96		81	68	**82**		96	88		103	**96**
	Final		87	82	66	**78**		90	88	93	98	**92**

SaO2 (%)	Infusion time	Pig 3	Pig 7	Pig 8	Pig 9	**Normal Reaction (mean)**		Pig 4*	Pig 5*	Pig 10*	Pig 11*	**Adverse Reaction (mean)**
	Basal	97	99	99	100	**99**		99	97	100		**99**
	10 min	97		99	100	**99**		99	97		94	**97**
	20 min	97		99	100	**99**		97	98			**98**
	30 min	96		99	100	**98**		97	98		93	**96**
	Final		99	99	100	**99**		96	98	100	93	**97**
Temp (ºC)	Infusion time	Pig 3	Pig 7	Pig 8	Pig 9	**Normal Reaction (mean)**		Pig 4*	Pig 5*	Pig 10*	Pig 11*	**Adverse Reaction (mean)**
	Basal	39	37	38	36	**37**		38	37	37		**37**
	10 min	39		38	36	**38**		38	37		37	**37**
	20 min	39		38	36	**38**		38	37			**37**
	30 min	39		38	36	**38**		38	37		38	**37**
	Final		37	38	36	**37**		38	37	37	38	**37**