A mathematical model of the role of aggregation in sonic hedgehog signalling

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A Mathematical Approach to Understanding the Role of Aggregation in Sonic Hedgehog Signalling

Supplementary Information

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S1   Model derivation

Our models describe the quantity of each potential aggregate size and their interactions in the formation of multimers. For the model equations described within this section we are required to set a 'largest' aggregate that can be formed. This aggregate is considered to consisting of n Shh proteins, where n is either given or is left arbitrary. We form nonlinear ordinary differential equations (ODEs) utilising the law of mass action.

S1.1   Multimerisation

We assume that multimerisation interactions occur in a pairwise manner; that is, multimers are formed via single interactions between multimers, monomers, or multimers and monomers. We do this as we consider the decreased likelihood of more than two Shh proteins, individually or as part of an aggregate, interacting within a local spatial location. Instead, we make the assumption that the concurrent interaction of multiple Shh aggregates and monomers can be viewed as a sequence of rapid, separate pairwise interactions.

An example is given below for a multimerisation system when $n = 3$ and as such is restricted to forming aggregates with as many as three Shh proteins to a single multimer. The nonlinear ODEs describing this process are given by Equation (1),

$$\frac{dx_1}{dt} = \overbrace{\alpha}^{\text{Source of monomers}} \underbrace{-2m_{1,1}x_1^2}_{\text{Formation of dimer}} \overbrace{-m_{1,2}x_1x_2}^{\text{Formation of trimer}} \underbrace{-\beta x_1}_{\text{Dispersal Term}}, \tag{1a}$$

$$\frac{dx_2}{dt} = m_{1,1}x_1^2 - m_{1,2}x_1x_2 - \beta x_2, \tag{1b}$$

$$\frac{dx_3}{dt} = m_{1,2}x_1x_2 - \beta x_3, \tag{1c}$$

where the initial conditions are given by

$$x_1(0) = 0, \qquad x_2(0) = 0, \quad \text{and} \quad x_3(0) = 0.$$

Equation (1) describes the rate of change in quantity of each size multimer. The monomer density, represented by $x_1 = x_1(t)$, increases with a constant rate α and represents an influx of Shh to the cell surface. The subsequent terms describe the removal of two monomers in the formation of a dimer and a single monomer in the formation of a trimer. The final terms of each equation describe the quantity-dependent dispersal of each monomer and multimer.

In the case of aggregates formed via multimerisation which are able to consist of up to n Shh protein, the governing equations are given by

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$$\frac{dx_1}{dt} = \overbrace{\alpha}^{\text{Source of monomers}} \underbrace{- \sum_{l=1}^{n-1} m_{1,l}^* x_1 x_l}_{\text{Monomer interactions with multimers}} \overbrace{- \beta x_1,}^{\text{Dispersal Term}} \tag{2a}$$

$$\frac{dx_i}{dt} = \overbrace{\sum_{a \geq b > 0}^{a+b=i} m_{a,b} x_a x_b}^{\text{All combinations that form a multimer with } i \text{ Shh}} \underbrace{- \sum_{l=1}^{n-i} m_{i,l}^* x_i x_l}_{\text{Formation of larger multimers from multimer with } i \text{ Shh}} \overbrace{- \beta x_i,}^{\text{Dispersal Term}} \quad \text{for } i = 2 : n, \tag{2b}$$

where the initial conditions are

$$x_l(0) = 0 \quad \text{for } l \in [1, n].$$

In Equation (2) we have the term $m_{a,b}^* = 2 \times m_{a,b}$ when $a = b$, and otherwise if $a \neq b$ we have $m_{a,b}^* = m_{a,b}$. As previously defined, binding rates are given by $m_{a,b}$ and describe the binding affinity for the interaction between a multimer (monomer if $a = 1$) with a associated monomers and a multimer with b (monomer if $b = 1$). We set the initial amount of Shh monomers and all aggregates (x_l for $l \in [1, n]$) to be equal to zero to represent the initial inactivity of Shh aggregation by the single cell we describe.

S1.2   Heparan Sulfate Proteoglycans

We assume that Shh-HSPG interactions occur in the following manner. Previously it was proposed that HSPGs would fulfill the role of acting as a 'scaffold' structure to promote the formation of large Shh aggregates. The work of Vyas and colleagues [1] in *D. melanogaster* suggests that Hh proteins undergo mandatory organisation events prior to interaction with HSPGs. We therefore made the assumption that Shh would be required to form multimers to bind HSPGs and. The publication by Vyas et al. notes that the disruption of multimerisation interactions leads to their inability to bind with HSPGs; to emulate this we do not allow monomeric Shh to bind HSPGs. Further, to explore the role of HSPGs in the formation of large aggregates and its function as a scaffold, we limit the size to which multimers are able to bind the structures for these interactions. The length of heparin chains may be one such factor that restricts the size of multimers that binds as is suggested by the structural insights reported by Whalen and colleagues [2].

We model HSPGs with a population of 'free' particles which bind with Shh multimers to form aggregates. For this, Shh is recruited to HSPGs as multimers to increase the size of the aggregate. To represent this mechanism we are required to include a system for the formation of small multimers with which HSPGs interact. Below we show the nonlinear

ODE model describing the formation of multimers with as many as three Shh monomers which are recruited by HSPGs to form aggregates with as many as 4 Shh proteins. This is represented in Equation 3.

$$\frac{dx_1}{dt} = \overbrace{\alpha}^{\substack{\text{Source of} \\ \text{Shh monomers}}} \underbrace{-2m_{1,1}x_1^2 - m_{1,2}x_1x_2}_{\text{Multimerisation terms}} \overbrace{-\beta x_1}^{\text{Monomer dispersal}}, \tag{3a}$$

$$\frac{dx_2}{dt} = m_{1,1}x_1^2 - m_{1,2}x_1x_2 \underbrace{-h_0x_2H_0}_{\substack{\text{Dimers recruited} \\ \text{to HSPG aggregates}}} -\beta x_2, \tag{3b}$$

$$\frac{dx_3}{dt} = m_{1,2}x_1x_2 - h_0x_3H_0 - \beta x_3, \tag{3c}$$

$$\frac{dH_0}{dt} = \overbrace{\delta}^{\substack{\text{Source of} \\ \text{free HSPGs}}} \overbrace{-h_0x_2H_0 - h_0x_3H_0}^{\substack{\text{Multimers binding} \\ \text{to free HSPGs}}}, \tag{3d}$$

$$\frac{dH_2}{dt} = \underbrace{h_0x_2H_0}_{\substack{\text{HSPG aggregate} \\ \text{formation}}} \underbrace{-h_2x_2H_2}_{\substack{\text{HSPG aggregate} \\ \text{dispersal}}} -\beta H_2, \tag{3e}$$

$$\frac{dH_3}{dt} = h_0x_3H_0 - \beta H_3, \tag{3f}$$

$$\frac{dH_4}{dt} = h_2x_2H_2 - \beta H_4, \tag{3g}$$

where the initial conditions are given by

$$x_a(0) = 0 \quad \text{for} \quad a = 1:3 \quad \text{and} \quad H_a(0) = 0 \quad \text{for} \quad a = 0:4.$$

In the HSPG model the included multimerisation system is as previously described, with x_i representing the population of multimers that consist of i Shh and x_1 describing the quantity of monomers. Analogously, we represent the population of HSPGs with i Shh bound by H_i, meaning H_0 represents those that are unoccupied by Shh. Of note, H_1 is not modelled as monomers are not able to bind HSPGs. The constant rate of dispersal is described by β and remaining terms are as discussed in Equations (3).

We next consider the derivation of a HSPG mechanism that is able to produce aggregates with as many as n bound. For this we include a system of Shh multimerisation to demonstrate interactions with HSPGs. Whilst we later elect a largest size for the constructed multimers based on literature, in this example we set the maximum multimer size to be arbitrary and given by p.

The system of nonlinear ODEs that describe the HSPG production of aggregates with up to n Shh monomers is given by

$$\frac{dx_1}{dt} = \overbrace{\alpha}^{\text{Monomer source}} - \underbrace{\sum_{l=1}^{p-1} m^*_{(1,l)} x_1 x_l}_{\substack{\text{Monomers bind to multimers} \\ \text{consisting of as many as } p-1 \text{ Shh}}} \overbrace{-\beta x_1}^{\text{Monomer dispersal}}, \tag{4a}$$

$$\frac{dx_i}{dt} = \underbrace{\sum_{\substack{j \geq k > 0}}^{j+k=i} m_{(j,k)} x_j x_k}_{\substack{\text{Formation of multimers} \\ \text{consisting of } i \text{ Shh}}} - \sum_{l=i}^{p-i} m^*_{(i,l)} x_i x_l \underbrace{-h_0 x_i H_0}_{\substack{\text{Multimer binds with} \\ \text{free HSPGs}}} \tag{4b}$$

$$\underbrace{-\sum_{c=2}^{n-i} h_c x_i H_c}_{\substack{\text{Multimer binds with} \\ \text{HSPG aggregate}}} \underbrace{-\beta x_i}_{\text{Multimer dispersal}}, \qquad \text{for } i = 2 : p,$$

$$\frac{dH_0}{dt} = \overbrace{\delta}^{\text{HSPG source}} \overbrace{-\sum_{i=2}^{p} h_0 x_i H_0}^{\substack{\text{Multimers binding} \\ \text{to free HSPGs}}}, \tag{4c}$$

$$\frac{dH_i}{dt} = \underbrace{h_0 x_i H_0}_{\substack{\text{Formation of HSPG} \\ \text{aggregates from free HSPGs}}} + \sum_{\substack{c \geq 2, \ p \geq d \geq 2}}^{c+d=i} h_c x_d H_c \tag{4d}$$

$$-\sum_{d=2}^{\min(n-i,p)} h_i x_d H_i \underbrace{-\beta H_i}_{\substack{\text{HSPG aggregate} \\ \text{dispersal}}}, \qquad \text{for } i = 2 : p,$$

$$\frac{dH_j}{dt} = \underbrace{\sum_{\substack{c \geq 2, \ p \geq d \geq 2}}^{c+d=j} h_c x_d H_c}_{\substack{\text{If the size of HSPG aggregates exceed that of multimers,} \\ \text{they cannot be formed by multimers binding to free HSPGs}}} - \sum_{d=2}^{\min(n-j,p)} h_j x_d H_j - \beta H_j, \qquad \text{for } j = p+1 : n, \tag{4e}$$

where the initial conditions are given by

$$x_a(0) = 0, \quad \text{for} \quad a = 1 : p,$$

and
$$H_a(0) = 0, \quad \text{for} \quad a = 0 : n.$$

Terms shown in Equation (4) are as defined in previous subsections. This includes the term $m^*_{a,b}$, which is defined by $m^*_{a,b} = 2 \times m_{a,b}$ when $a = b$, and otherwise if $a \neq b$ we have $m^*_{a,b} = m_{a,b}$.

S1.3 Lipoproteins

We model lipoprotein interactions similar to that of HSPGs in that particles act as a surface with which Shh binds in the formation of aggregates. Currently, the understanding of lipoprotein-Shh interactions remains mostly unclear and there is limited experimental evidence that indicates how the mechanism may operate. We therefore make a number of assumptions based on how we interpret these events to occur. A central assumption we make is that Shh will be recruited to and binds lipoproteins in singular interactions. This means we make the presumption that Shh cannot bind lipoproteins after forming into a multimer. We make this distinction as some researchers have suggested that Shh utilises its lipid heads to bind the phospholid monolayer of lipoproteins [3]; the formation of multimers would most likely occur such that Shh directs its lipid heads into the core, which would negate the potential to associate with lipoproteins. In addition, the formation of multimers would, as is hypothesised in previous literature discussions [4, 5], resolve Shh of its hydrophobicity without any requirement of lipoprotein interaction. In a preliminary example we describe the formation of lipoprotein aggregates that bind as many as three Shh monomers. This is represented by the system of nonlinear ODEs given in Equation (5),

$$\frac{dx_1}{dt} = \overbrace{\alpha}^{\text{Monomer source}} \underbrace{-k_1 x_1 l_0}_{\text{Monomers bind to free lipoproteins}} \overbrace{-k_2 x_1 l_1 - k_3 x_1 l_2}^{\text{Monomers bind lipoprotein aggregates}} \underbrace{-\beta x_1}_{\text{Monomer dispersal}}, \tag{5a}$$

$$\frac{dl_0}{dt} = \overbrace{\gamma}^{\text{Lipoprotein source}} -k_1 x_1 l_0, \tag{5b}$$

$$\frac{dl_1}{dt} = k_1 x_1 l_0 \quad - k_2 x_1 l_1 \quad \overbrace{-\beta l_1}^{\text{Lipoprotein dispersal}}, \tag{5c}$$

$$\frac{dl_2}{dt} = k_2 x_1 l_1 \quad - k_3 x_1 l_2 \quad - \beta l_2, \tag{5d}$$

$$\frac{dl_3}{dt} = k_3 x_1 l_2 \quad - \beta l_3, \tag{5e}$$

where the initial conditions are given by

$$x_1(0) = 0, \quad l_0(0) = 0, \quad l_1(0) = 0, \quad l_2(0) = 0 \quad \text{and} \quad l_3(0) = 0.$$

In the system of equations given by Equation (5) a number of terms are as previously defined in Equation (1); the constant addition of Shh monomers is represented by α and β describes the rate of dispersal of aggregates from the cell surface. In addition, we use γ to represent the constant addition of free lipoproteins that are not bound by Shh.

We next show the system of equations that describes the formation of lipoprotein aggregates that are able to bind as many as n Shh monomers. This is given by the following system of nonlinear ODEs,

$$\frac{dx_1}{dt} = \overbrace{\alpha}^{\text{Monomer source}} \underbrace{-k_1 x_1 l_0}_{\substack{\text{Monomer binding} \\ \text{to free lipoproteins}}} \overbrace{-\sum_{d=1}^{n} k_{d+1} x_1 l_d}^{\text{Monomers bind lipoprotein aggregates}} \underbrace{-\beta x_1}_{\text{Monomer dispersal}}, \tag{6a}$$

$$\frac{dl_0}{dt} = \overbrace{\gamma}^{\text{Lipoprotein source}} -k_1 l_0 x_1, \tag{6b}$$

$$\frac{dl_i}{dt} = \overbrace{k_i l_{i-1} x_1}^{\substack{\text{Formation of lipoprotein} \\ \text{aggregate with } i \text{ Shh bound}}} \underbrace{-k_{i+1} x_1 l_i}_{\substack{\text{Lipoprotein:Shh monomer} \\ \text{association}}} \overbrace{-\beta l_i}^{\substack{\text{Lipoprotein} \\ \text{aggregate dispersal}}}, \quad \text{for } i = 1 : n-1, \tag{6c}$$

$$\frac{dl_n}{dt} = k_n x_1 l_{n-1} - \beta l_n, \tag{6d}$$

where the initial conditions are given by

$$x_1(0) = 0, \quad l_0(0) = 0 \quad \text{and} \quad l_a(0) = 0 \quad \text{for} \quad a = 1 : n.$$

S2 Estimating parameter values

Currently, the amount of available data that would be essential to inform model parameters is largely insufficient. Nonetheless, we are able to effectively limit the parameter space by considering previous estimates made by other and qualitative data for aggregate production. We first note that as our model considers a single cell we make the initial assumption that 800 Shh are produced per minute, which follows from an estimate made by Dillon et al. for a Shh signalling model [6]. This gives the rate of monomer production per day (α) to be 1,152,000 proteins per day. Our remaining parameters were estimated such that each mechanism was appropriately represented and contributed in conformity with the literature. First, we assumed sensible upper and lower bounds for the expression of HSPGs and lipoproteins and we initially based this on the ratio of monomer to particle that is introduced over the 24-hour simulation period. We reason that the expression of a particular particle, either HSPG of lipoprotein, cannot not exceed that of Shh monomers. We found that an excessive rate of particle introduction would drive a particular mechanism to dominate aggregate production and leave the remaining mechanisms to become more redundant. On the other hand, it is equally important that the source of particles

is not too low and mechanisms are able to provide a sufficient contribution to aggregate production, which would otherwise be in disagreement with literature that indicates each mechanism has an important role in aggregate production. We observed that if the rate of introduction for HSPG or lipoprotein particles was too low the aggregates produced via the relevant mechanisms would always be cell-associated in the largest sizes. We reason that this instance is largely unlikely to be biologically feasible and thus set a lower bound such that aggregates would be produced with a greater variation in the amount of Shh associated.

We considered it important to utilise values for the introduction of HSPGs and lipoproteins that would not greatly impact multimerisation and would also not excessively diminish the cell-associated monomer population. We also initially chose to keep the binding rates of multimerisation, lipoproteins and HSPGs within a similar order of magnitude and with values that were indicated by the data fitting results. We obtained a range of values for the rate of aggregate dispersal by data fitting both the cell-associated and dispersal data reported by [7] simultaneously, but noted the range of values was broad. We found that varying the rate of aggregate dispersal does not significantly impact the rate of aggregate production and the distribution of cell-associate aggregates is not visibly affected unless it is almost 100-fold larger.

We completed a sensitivity analysis to refine our values further and again qualitatively compared our simulation results at 24 hours with that of Koleva and colleagues [7]. The parameter values we used are given in S1 Table.
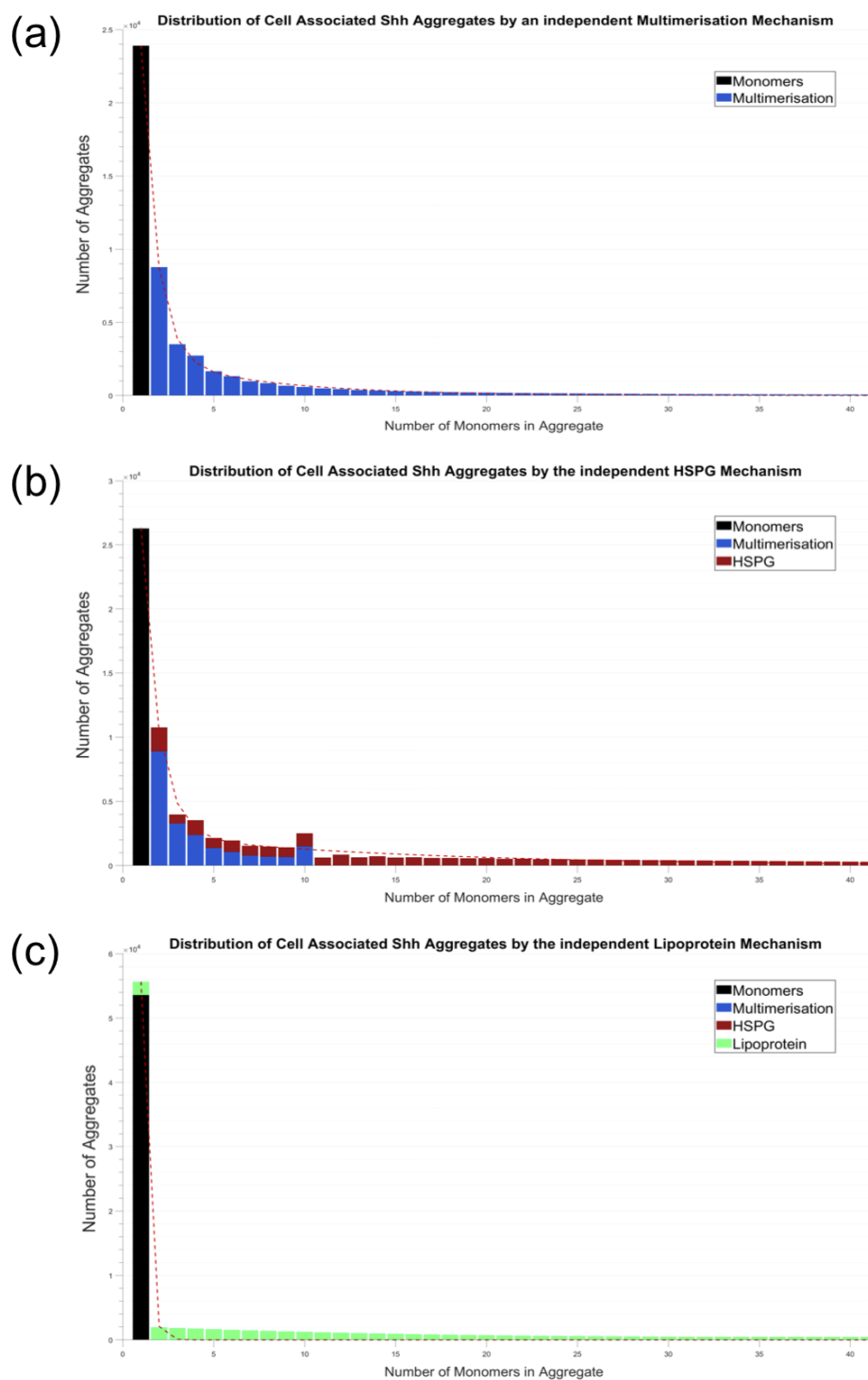
Parameter	Definition	Value	Source
α	Source of monomers	1.152×10^6 monomers/day	[6]
δ	Source of HSPGs	4.60×10^4 particles/day	Estimate.
γ	Source of lipoproteins	6.00×10^4 particles/day	Estimate.
β	Rate of dispersal	0.75 day^{-1}	Estimate.
$m_{a,b}$	Multimerisation binding rate	6.00×10^{-4} (multimer day)$^{-1}$	Estimate.
h_i	Multimer to HSPG binding rate	4.00×10^{-4} (multimer day)$^{-1}$	Estimate.
k_i	Monomer to lipoprotein binding rate	5.00×10^{-4} (protein day)$^{-1}$	Estimate.

S1 Table: Parameters for the aggregation model.

We reason that our chosen values are suitable for each mechanism. The source of HSPGs in respect to monomer availability means that the particle to monomer ratio is ∼25:1. Larger sources of HSPGs were found to be more disruptive to multimerisation due to a greater rate of recruitment. For our lipoprotein source value, we note that that the ratio for the expression of lipoproteins to monomers is 19.2:1 This expression allows the lipoprotein to contribute a reasonable degree of competition for monomers with the multimerisation mechanism, but does not cause lipoproteins to excessively dominate aggregate production.

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S3   Individual mechanism distributions

We investigated the distribution at 24-hours when each mechanism functions independent of the others. Using the parameters described in S1 Table we simulate aggregate formation whilst opposing mechanism interactions are disabled, with the exception of HSPGs which requires a system for the formation of small multimers (as many as 10 monomers to an aggregate) with which it binds. The distributes produced are shown in S1 Fig.

(a)

Distribution of Cell Associated Shh Aggregates by an independent Multimerisation Mechanism

(b)

Distribution of Cell Associated Shh Aggregates by the independent HSPG Mechanism

(c)

Distribution of Cell Associated Shh Aggregates by the independent Lipoprotein Mechanism

S1 Fig: Shh aggregate formation as a result of each individual mechanism: Here, aggregates are formed via **(a)** multimerisation, **(b)** HSPG and **(c)** lipoprotein recruitment in absence of the remaining two mechanisms. We allow the formation of small multimers in the individual HSPG model to allow recruitment to occur. Multimerisation in this model is however restricted and cannot consist of more than 10 Shh monomers. Simulations shown are at 24 hours.

S4 Steady-state distribution
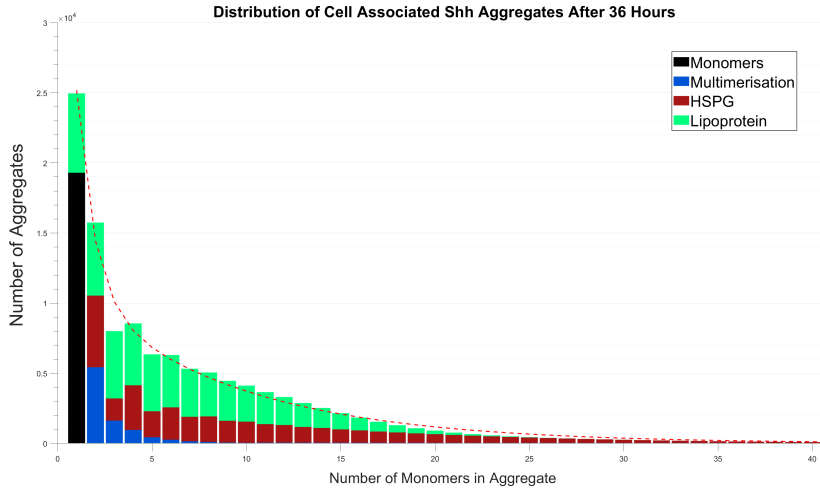
S2 Fig: Cell associated Shh distribution at steady state: Shh aggregate formation as a result of multimerisation, HSPG and lipoprotein association: Tables indicate the percentage of each mechanism responsible for forming the respective size aggregate in terms of the total number of Shh protein monomers and aggregates formed. Simulation shown is at steady state (∼36 hours).
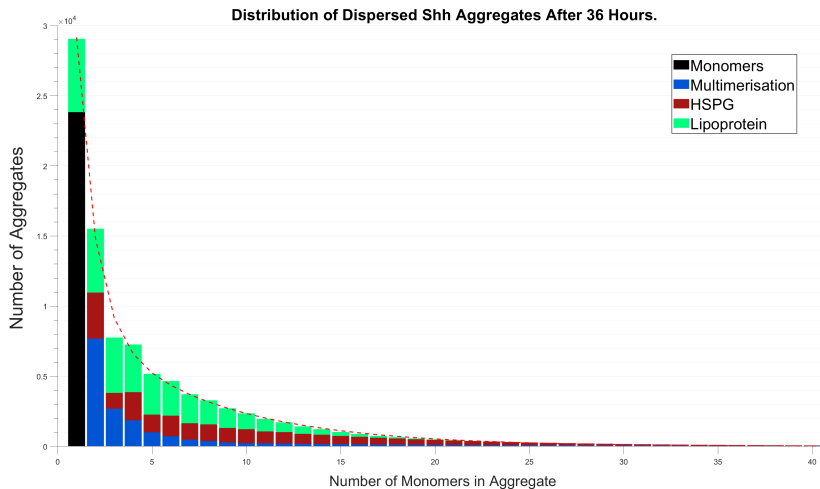
S3 Fig: Dispersed Shh distribution at steady state: Shh aggregate formation as a result of multimerisation, HSPG and lipoprotein association: Tables indicate the percentage of each mechanism responsible for forming the respective size aggregate in terms of the total number of Shh protein monomers and aggregates formed. Simulation shown is at steady state (∼36 hours).

	1	2	3	4	5	6	7	8	9	10	
Monomers	16.31	-	-	-	-	-	-	-	-	-	
Multimers	-	4.59	1.37	0.81	0.37	0.22	0.12	0.08	0.05	0.03	
HSPGs	0.00	4.32	1.34	2.69	1.57	1.95	1.48	1.54	1.31	1.28	
Lipoproteins	4.79	4.41	4.05	3.73	3.43	3.16	2.90	2.65	2.41	2.17	
	11	12	13	14	15	16	17	18	19	20	
Monomers	-	-	-	-	-	-	-	-	-	-	
Multimers	0.04	0.04	0.04	0.04	0.04	0.04	0.03	0.03	0.03	0.03	
HSPGs	1.12	1.06	0.95	0.89	0.80	0.75	0.68	0.63	0.57	0.52	
Lipoproteins	1.93	1.69	1.44	1.21	0.98	0.77	0.59	0.43	0.31	0.21	
	21	22	23	24	25	26	27	28	29	30	
Monomers	-	-	-	-	-	-	-	-	-	-	
Multimers	0.03	0.03	0.03	0.03	0.03	0.02	0.02	0.02	0.02	0.02	
HSPGs	0.48	0.44	0.40	0.36	0.33	0.30	0.27	0.24	0.22	0.20	
Lipoproteins	0.14	0.09	0.06	0.03	0.02	0.01	0.01	0.00	0.00	0.00	
	31	32	33	34	35	36	37	38	39	40	> 40
Monomers	-	-	-	-	-	-	-	-	-	-	-
Multimers	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	1.52
HSPGs	0.18	0.16	0.14	0.12	0.11	0.10	0.09	0.08	0.07	0.06	0.36
Lipoproteins	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

S2 Table: Percentage breakdown of mechanisms that form the steady-state cell associated Shh aggregate distribution as shown in S2 Fig. The percentage of each mechanism responsible for forming the respective size aggregate in terms of the total number of Shh protein monomers and aggregates formed.

	1	2	3	4	5	6	7	8	9	10	
Monomers	24.33	-	-	-	-	-	-	-	-	-	
Multimers	-	7.84	2.74	1.90	1.02	0.73	0.49	0.39	0.28	0.22	
HSPGs	0.00	3.35	1.15	2.05	1.28	1.50	1.19	1.21	1.06	1.02	
Lipoproteins	5.34	4.65	4.03	3.47	2.97	2.52	2.11	1.75	1.44	1.16	
	11	12	13	14	15	16	17	18	19	20	
Monomers	-	-	-	-	-	-	-	-	-	-	
Multimers	0.22	0.21	0.19	0.18	0.16	0.15	0.14	0.13	0.12	0.11	
HSPGs	0.86	0.83	0.72	0.67	0.60	0.55	0.49	0.44	0.40	0.36	
Lipoproteins	0.92	0.71	0.54	0.40	0.29	0.21	0.14	0.09	0.06	0.04	
	21	22	23	24	25	26	27	28	29	30	
Monomers	-	-	-	-	-	-	-	-	-	-	
Multimers	0.10	0.10	0.09	0.09	0.08	0.08	0.07	0.07	0.07	0.06	
HSPGs	0.32	0.29	0.25	0.23	0.20	0.18	0.16	0.14	0.12	0.11	
Lipoproteins	0.02	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
	31	32	33	34	35	36	37	38	39	40	>40
Monomers	-	-	-	-	-	-	-	-	-	-	-
Multimers	0.06	0.06	0.06	0.05	0.05	0.05	0.05	0.05	0.04	0.04	1.82
HSPGs	0.09	0.08	0.07	0.06	0.05	0.05	0.04	0.03	0.03	0.03	0.14
Lipoproteins	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

S3 Table: Percentage breakdown of mechanisms that form the steady-state dispersed Shh aggregate distribution as shown in S3 Fig. The percentage of each mechanism responsible for forming the respective size aggregate in terms of the total number of Shh protein monomers and aggregates formed.

# S5 Full view of distribution figure axis
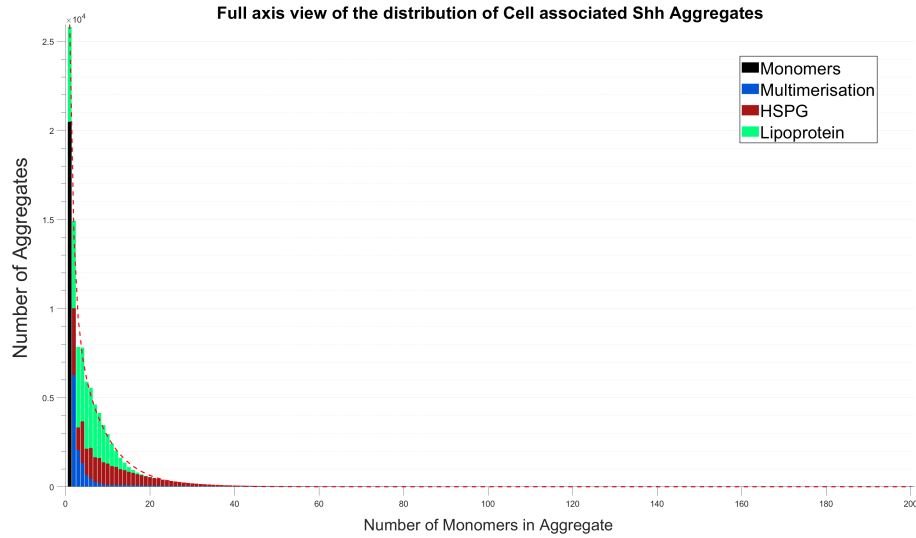
S4 Fig: Full axis view of the Cell Associated Shh distribution. Figures show the full distribution of aggregates that consist of up to 200 monomers. Simulation shown is at 24 hours.
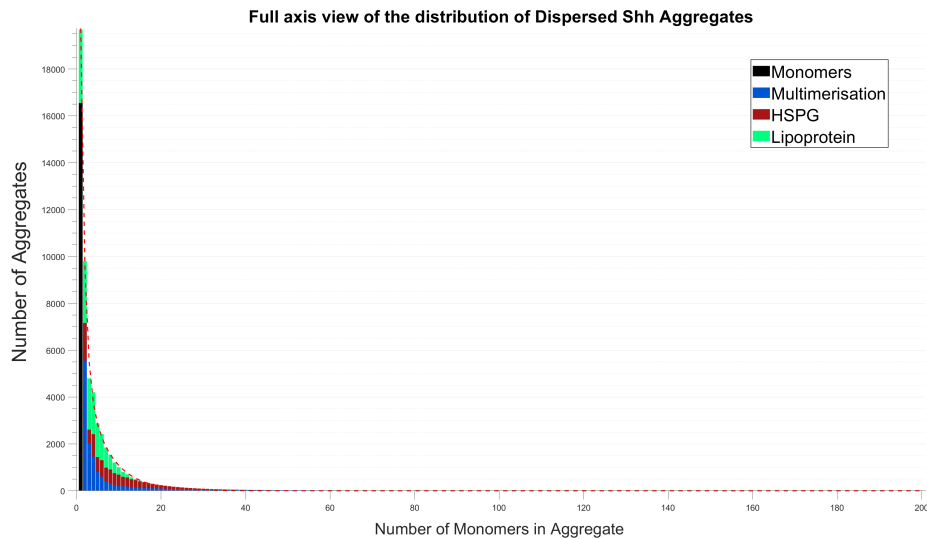
S5 Fig: Full axis view of the Dispersed Shh distribution. Figures show the full distribution of dispersed Shh aggregates that consist of up to 200 monomers. Simulation shown is at 24 hours.

S6 Monomer dispersal removed
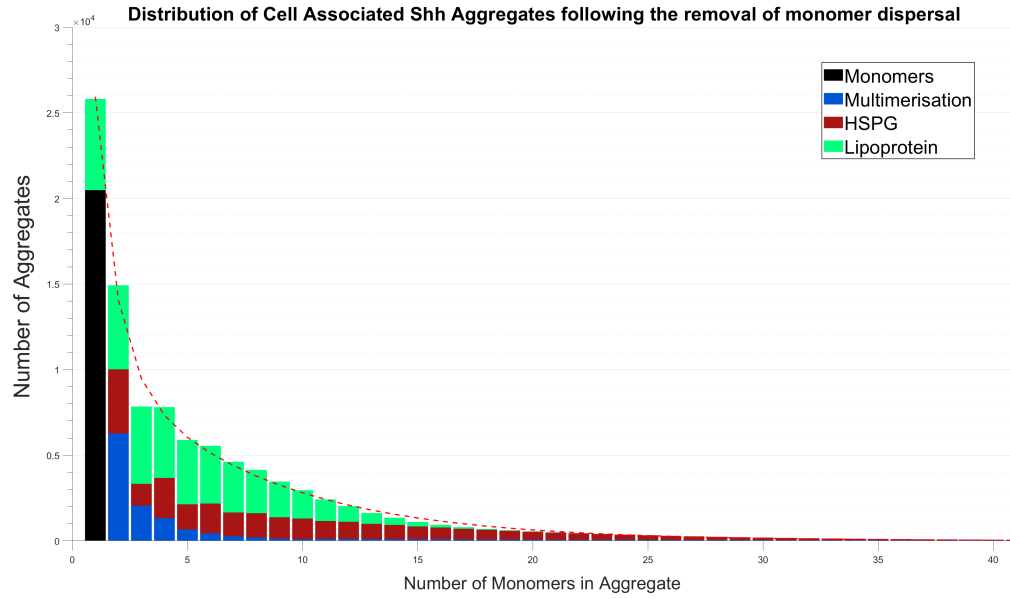
S6 Fig: Cell associated Shh aggregate formation following the removal of monomer dispersal: Simulation shown is at 24 hours.

S7 Sensitivity analysis

We conducted a sensitivity analysis to observe the effect that variation in certain parameters has on aggregate production. We found that increasing the source values for HSPGs or lipoproteins benefited the respective mechanisms by increasing the quantity of aggregates that could be produced by each process. This would consequently increase the competition within the mechanism; an increased number of aggregates recruit from the same population, depleting it at a greater rate and ultimately leads to a reduction in the average number of Shh that associates into the respective aggregates. In both scenarios multimer formation would be impeded. This is because an increase in lipoproteins would subsequently broaden their competition for Shh monomers and an increased quantity of HSPG aggregates would promote a greater rate of multimer recruitment. However, whereas an increase in the HSPG source rate would benefit lipoproteins by reducing multimer formation, an increase in lipoproteins would hinder HSPG aggregation, which is a result of the decreased rates of multimerisation.

Changes to the rate of dispersal was summarised in the main text and the distribution produced following the complete removal of dispersal was given in Fig 6. Increasing the rate of dispersal severely disrupted the overall production of aggregates, and impacted multimerisation least. This is most likely due to the increased removal of free particles by which HSPGs and lipoproteins are required to utilise in the formation of aggregates. Multimerisation is distinct from this and instead is not reliant on these particles. A reduction in rate of dispersal leads to an opposite effect. Aggregate production by the lipoprotein mechanism was dominant when compared to the remaining mechanisms. Because of this, multimer production was significantly inhibited due to the greater competition for Shh monomers by lipoprotein recruitment. The increased retaining of HSPGs at the cell surface as a result of a reduced sink is further detrimental to multimer formation. This is because the increased number of HSPGs cause a greater rate of multimer recruitment. However, as multimers are formed at reduced rates and with a lesser monomer availability as a result of the aforementioned outcomes, the HSPG mechanism has an overall minimal benefit when dispersal rates are increased.

Increasing the rate of multimerisation enhances multimer production and consequently HSPG aggregate formation. The lipoprotein mechanism is subject to a greater degree of competition for monomers from multimerisation and thus aggregate production with lipoproteins is impeded. An increased rate of HSPG production benefits the respective mechanism in forming greater sizes but reduces multimer production due to increased recruitment. This however enhances the formation of aggregates by lipoproteins, which profits from reduced competition by multimers. Lastly, increasing the rate of lipoprotein aggregate formation disrupts both the HSPG and multimerisation mechanism through increased competition for monomers and reducing multimer production.
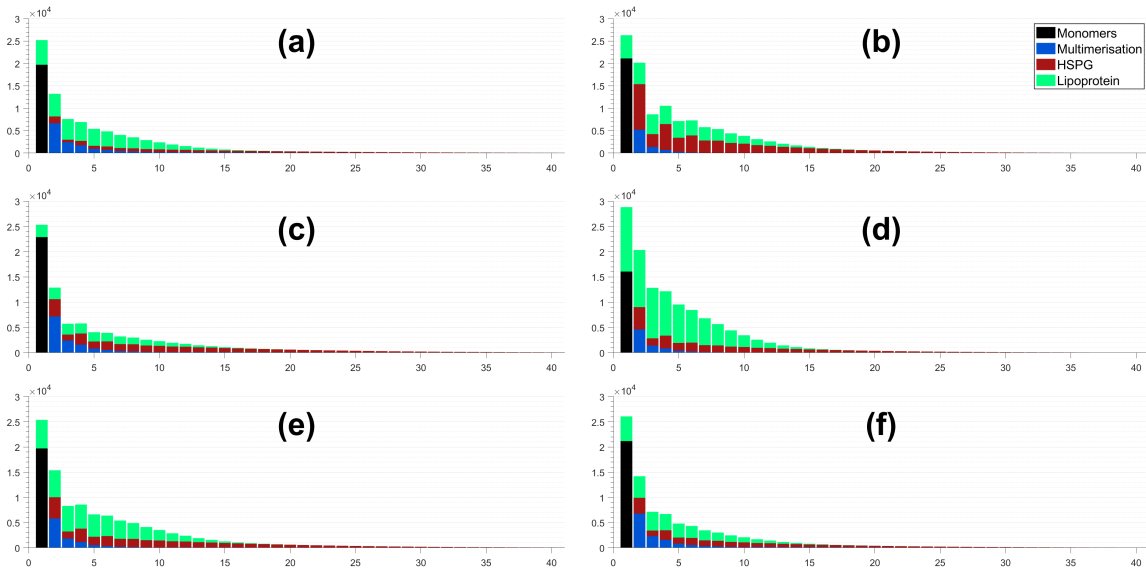
S7 Fig: Shh aggregate distributions with: (a) half the source of HSPGs; (b) doubled source of HSPGs; (c) half the source of lipoproteins; (d) doubled source of lipoproteins; (e) half rate of dispersal; and (f) doubled rate of dispersal.
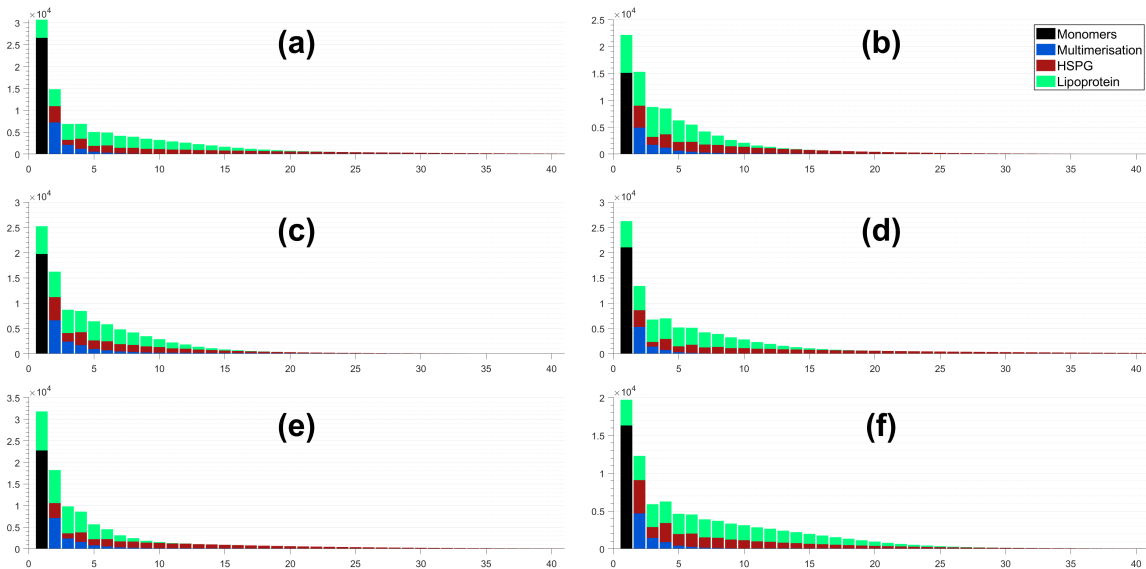
S8 Fig: Shh aggregate distributions with: (a) half the rate of multimerisation; (b) doubled rate of multimerisation; (c) half the rate of HSPG binding; (d) doubled rate of HSPG binding; (e) half the rate of lipoprotein binding; and (f) double the rate of lipoprotein binding.

S8   Diffusion coefficient approximation

Produced aggregates are likely to have considerably differing rates of diffusion between the mechanisms. In addition, the continued binding of Shh will impact aggregates, and the degree of this will vary dependent on the mechanism by which the aggregate is produced. In the following we seek to explore this using estimates and available literature sources.

To calculate diffusion coefficient for Shh aggregates produced via multimerisation, HSPG and lipoprotein recruitment, we make assumptions for the structure by which each are made. In the follow discussions we utilise a diameter for Shh monomer of 4 nm, which is as noted by Koleva and colleagues [7].

Multimerisation

To calculate estimations for Shh multimer diffusion coefficients we first consider approximations for a Shh monomer, a multimer consisting of 6 Shh proteins (hexamer) and a multimer that consists of 36 monomers (36-mer). We aim to model each as a sphere and calculate the diffusion coefficient using the Stokes-Einstein equation given by Equation 7, for which we are required to estimate the radius of each. For monomers we assumed that a Shh protein can be modelled as a sphere which has a radius of 2 nm. We next assume that hexamers can be considered as comprising of equally arranged monomers forming as sphere, as depicted in S9(a) Fig. This structure would therefore have a radius that is equal to the diameter of a monomer, which is 4 nm. For a 36-mer we continue this approach and assume that the larger aggregate can be viewed as a composition of Shh hexamers that are arranged equally around a center, as is shown in S9(b) Fig. This multimer is therefore assumed to have the radius of two Shh monomer diameters (8 nm).

We calculated the diffusion coefficient of a monomer, hexamer and 36-mer by using the Stokes-Einstein equation, which approximates the diffusion of spherical particles through a liquid medium at a constant temperature. The Stokes-Einstein equation is given by,

$$D = \frac{kT}{6\pi\eta r},\tag{7}$$

where k is the Boltzmann's constant, T is the temperature in Kelvin, η is the viscosity of the liquid medium and r is the radius of the spherical particle. For our calculations we assume that the fluid through which Shh aggregates diffuse has a viscosity equivalent to water, which, at a temperature of 298.15 Kelvin (25° Celsius), is 8.9×10^{-4} Pa·.

Using the curve fitting toolbox (cftool) in MATLAB [8] we find the coefficients for a quadratic equation that is sufficient to fit the diffusion coefficients calculated for the monomer, hexamer and 36-mer. This allows us to estimate the coefficients for remaining multimers.
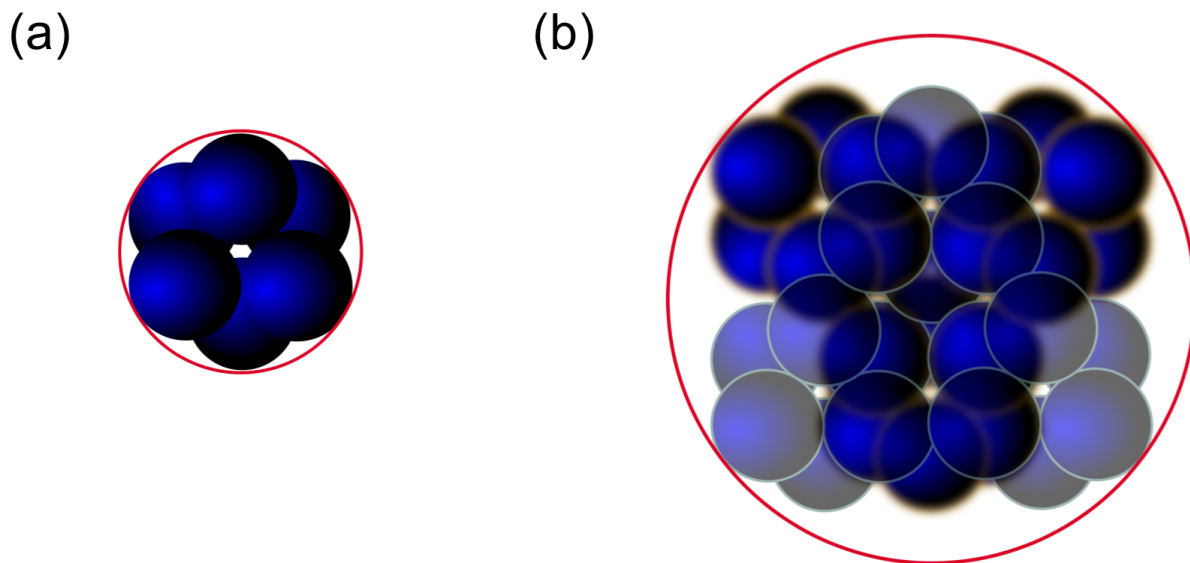
S9 Fig: Diagram of Shh multimers: We assume a hexamer **(a)** can be modelled as a sphere with the radius of a Shh monomer and a 36-mer **(b)** can be modelled as a sphere that has a radius equal to the diameter of a hexamer.
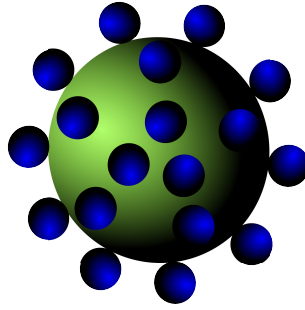
Lipoproteins

The particular class of lipoproteins that is likely to bind Shh for transport has not been identified. For our calculations we considered the binding of low-density lipoproteins (LDL) as this subspecies is sufficiently large to be occupied by the amounts of Shh binding that we consider and still be comparable in overall size to the opposing mechanisms.

To calculate the diffusion coefficient of lipoprotein aggregates we make the assumption that the binding of Shh to lipoproteins creates a 'layer' of Shh monomers on the surface. Large amounts of Shh binding to lipoproteins creates a solid layer around the particle which creates an overall larger sphere with a greater radius. Shh binding to lipoproteins is depicted in S10 Fig.

From Tindall et al. [9] we have that the radius of an LDL particle is 10 nm and therefore an LDL bound by a maximum amount of Shh has a radius of 14 nm (radius added to the diameter of a Shh monomer).

Using the Stokes-Einstein equation (Equation (5)) we are able to calculate an approximate diffusion coefficient for both a lipoprotein that is not bound by Shh and one that is bound with a maximum amount of Shh. By assuming that the latter estimate corresponds to the binding of 40 Shh monomers, we utilise a data fitting via MATLAB [8] to estimate the diffusion coefficients for remaining lipoprotein aggregate sizes.
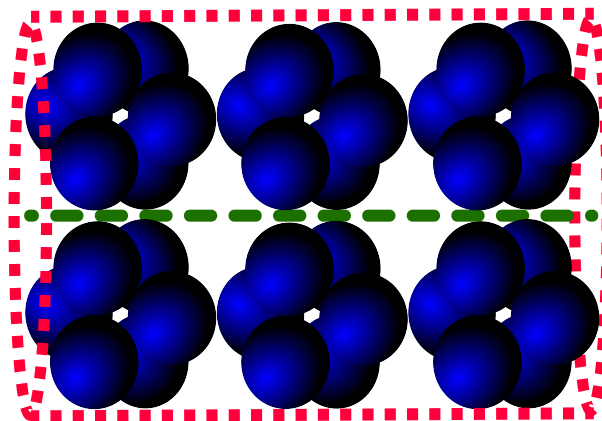
S10 Fig: Diagram of Shh aggregation by association with lipoproteins:
Here we assume that Shh monomers bind a lipoprotein individually and contribute
to form a 'layer' upon the particle.

HSPGs

Creating an assumption for the shape of Shh-HSPG aggregates is especially difficult due
to the ambiguous role for which it promotes Shh aggregate formation. The assumption we
elect to make follows in part from the chain-like structures that were considered by Whalen
and colleagues [2]. As we depict in S11 Fig, HSPG aggregates are viewed as 'tubular'
chain-like structures. We follow a similar approach as with the mechanisms described
previously and assume that Shh bound HSPGs are in the form of various hexamers which
bind opposing sides of a HSPG chain. In this sense a HSPG aggregate that is bound by
36 Shh proteins is to be modelled as a cylinder consisting of 6 hexamers divided across
opposing sides of the heparin chain. The radius of this cylinder will be be equal to the
diameter of a hexamer which we discussed and calculated above. The length of cylinder
can also be found to be equal to the diameter of 3 hexamers.

This process was applied to HSPG aggregates with 12, 24 and 36 Shh monomers associ-
ated, which meant cylinder lengths of 8nm, 16nm and 24nm respectively, to calculate an
approximate volume. We also considered a HSPG aggregate with a sole hexamer bound
and assumed the volume would be equal to that if it were not bound to a HSPG. With
these volumes we calculated approximations to the diffusion coefficient using Equation
(5), and applied Matlab's curve fitting toolbox to estimate the values for the remaining
HSPG aggregate sizes.

We arrive at the following estimates for the diffusion coefficient for the aggregates produced
by different mechanisms and composition of monomers.

S11 Fig: Diagram of Shh aggregation by association with HSPGs: We assume that Shh multimers bind HSPGs as hexamers to form a structure that can be modelled as a cylinder.
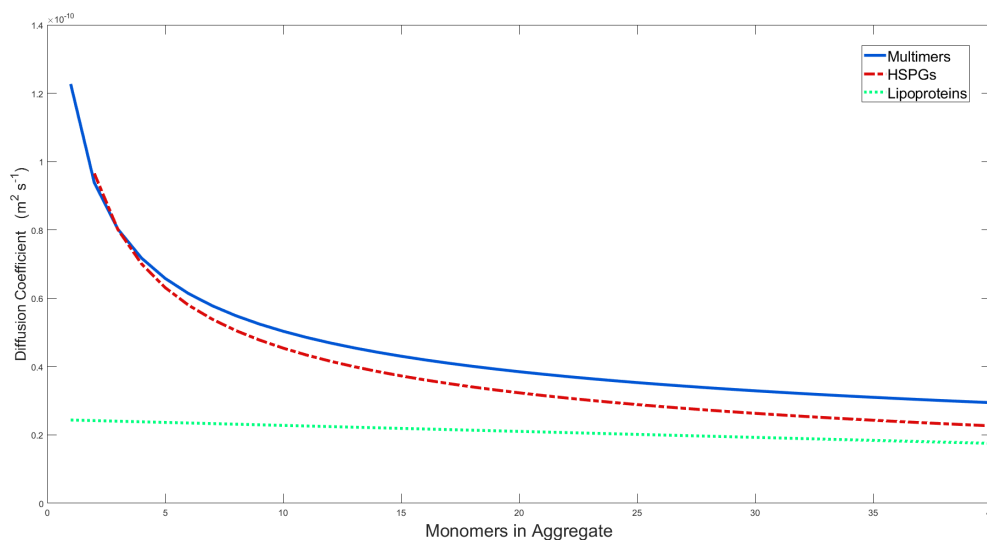
S12 Fig: Approximated diffusion coefficients for the aggregates produced by multimerisation, HSPG and lipoprotein recruitment.

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