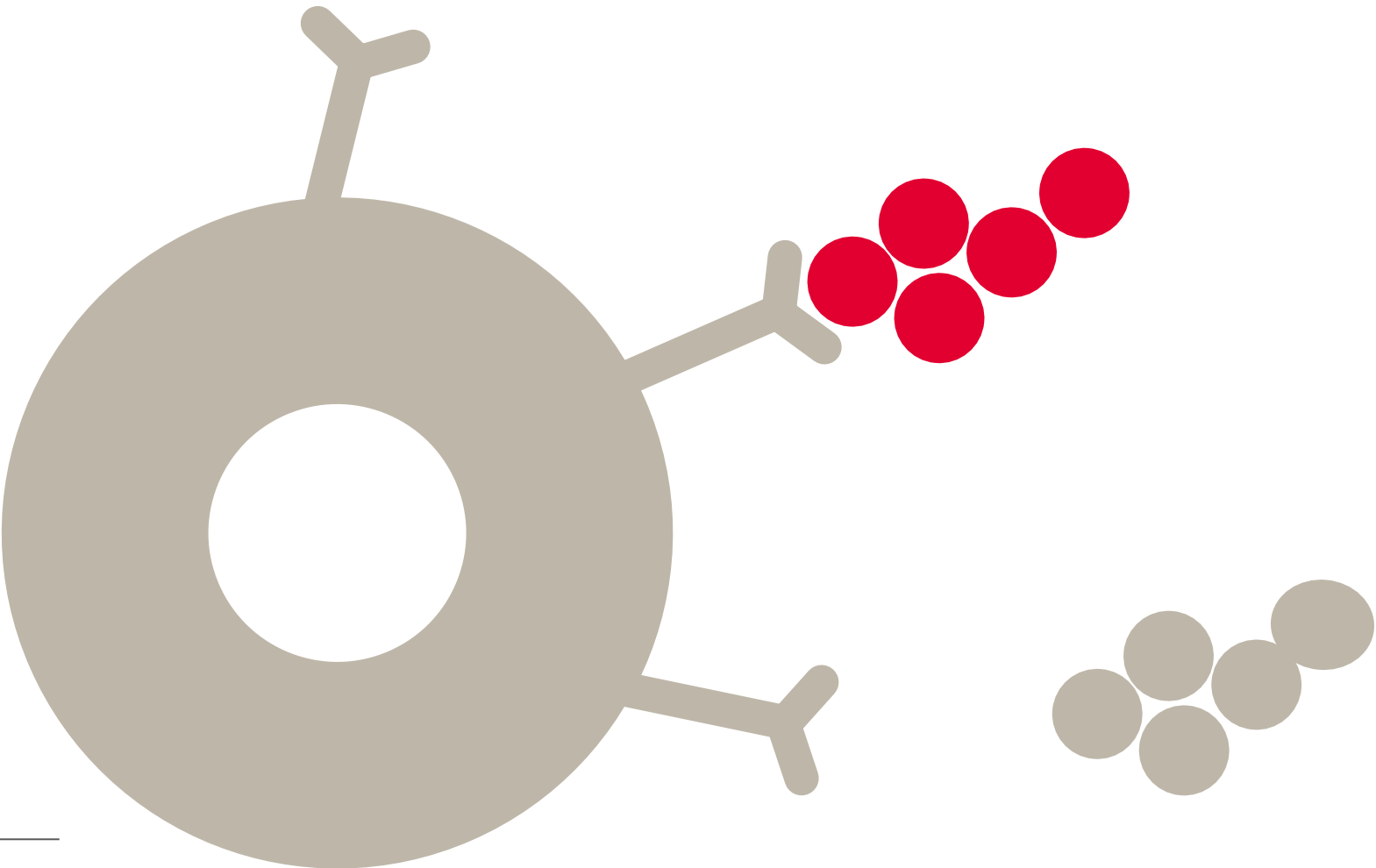
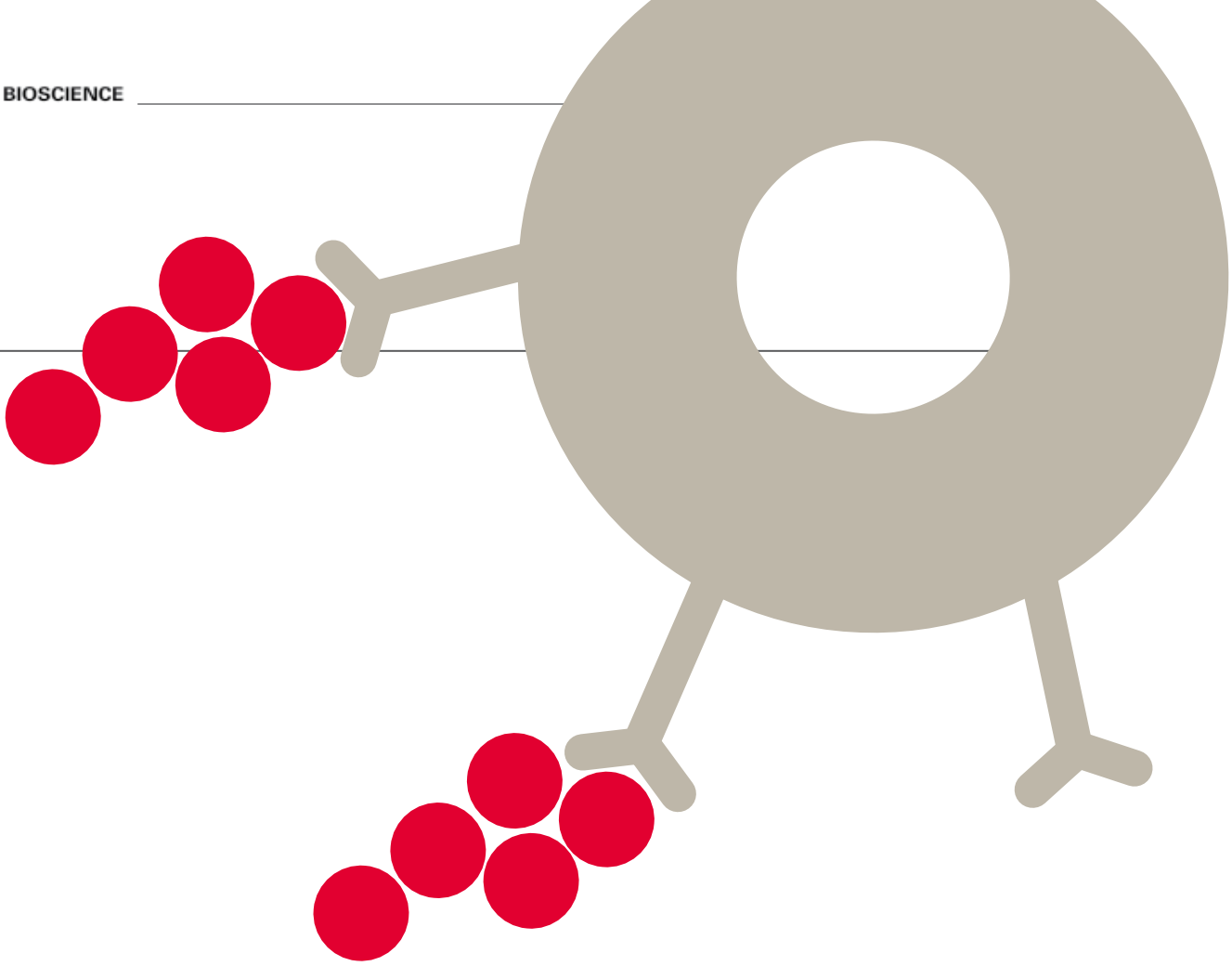




TOSOH

AFFINITY
CHROMATOGRAPHY



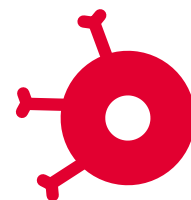
AFC AFFINITY CHROMATOGRAPHY

AFC PRODUCTS

- Toyopearl Resins for Protein A Affinity Chromatography - NEW
 - Toyopearl Resins for Affinity Ligand Coupling
 - Activated
 - Reactive
 - Toyopearl Resins with Group Specific
 - ToyoScreen Process Development Columns

TOSOH FACT

Tosoh Bioscience GmbH offers workshops on chromatography in process development & production. These three day courses deliver a comprehensive background to bioprocess chromatographic purification as an integrated process technique. The workshops provide a balance of effective presentations and practical hands-on experience with process scale and methods development equipment under the guidance of qualified and knowledgeable Tosoh Bioscience technical experts. Every year more than 30 participants use this opportunity to broaden or refresh their chromatographic and downstream processing knowledge.





AFFINITY CHROMATOGRAPHY

TOYOPEARL AFFINITY RESINS FOR PROCESS SCALE CHROMATOGRAPHY

There are many custom designed affinity ligands available to the chromatographer. Toyopearl affinity chromatography resins are functionalized with chemically active groups or group-specific ligands. Resins with activated functional groups are ready to directly couple a protein or other ligand. Resins with reactive groups require carbodiimide coupling or reductive amination to achieve a stable covalent linkage. The latest development in Toyopearl affinity resins is the high capacity Protein A affinity resin Toyopearl AF-rProtein A-650F developed to increase productivity in antibody purification.

PRESSURE-FLOW CHARACTERISTICS AND PHYSICAL/CHEMICAL STABILITY

Toyopearl resins remain dimensionally stable within wide extremes of pH and ionic strength. Moreover, the semi-rigid Toyopearl particles do not distort under flow rates that generate up to 3 bar pressure. These properties of the resins combined with the narrow particle size distributions result in superior pressure-flow characteristics for the packed Toyopearl bed. Linear velocities of 300 - 500 cm/h generate a pressure of between 1 and 2 bar in a 20 cm length bed. Achievement of high linear velocities at relatively low pressure enables high throughput production scale chromatography using equipment with moderate pressure limitations. Sanitization or cleaning may be conducted with up to 0.5 mol/L NaOH or 0.5 mol/L HCl depending upon the ligand. In affinity chromatography, in particular, the choice of cleaning agent will be largely dependent upon the chemical stability of the ligand, rather than that of the base resin.

➤ **TABLE I**

Protein A Affinity	Activated resin	Reactive resin	Group specific
Protein A	AF-Tresyl AF-Epoxy	AF-Amino AF-Carboxy AF-Formyl	AF-Blue HC AF-Red AF-Chelate AF-Heparin HC

➤ FEATURES

- Active, reactive and group specific resins
- New high capacity AF-rProtein A resin
- Standard 1.000 Å pore size
- Porous, hydrophilic polymer matrix
- High mechanical stability

TOYOPEARL RESIN FOR PROTEIN A AFFINITY CHROMATOGRAPHY

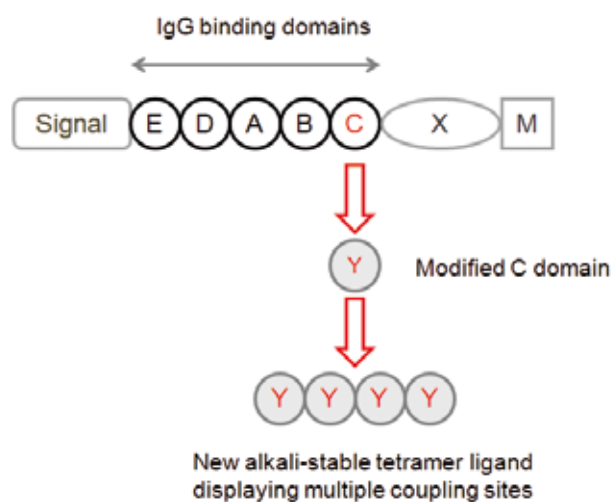
Toyopearl AF-rProtein A-650F is an affinity resin designed for efficient and robust large-scale purification of monoclonal antibodies. A newly developed recombinant protein A ligand (*E. coli*) was linked to the well proven methacrylic polymer backbone of Toyopearl media. The ligand is derived from one of the IgG-binding domains of the *staphylococcus aureus* protein A (Figure 1). Its amino acid sequence was optimized in order to increase its stability towards alkaline solutions.

Multipoint attachment of the ligand to the Toyopearl matrix further enhances chemical and thermal stability of the resin. In practice this pays off for a low level of protein A leaching and also for a high resistance to alkaline solutions employed in cleaning-in-place (CIP) procedures.

Toyopearl AF-rProtein-650F binds human and mouse immunoglobulin G, and Fab fragments.

➤ **FIGURE 1**

RECOMBINANT PROTEIN A DERIVED LIGAND



➤ BENEFITS

- Broad range of applications
- Efficient antibody purification
- High capacity for large biopolymers
- Suitable for laboratory and process scale purifications
- Constant bed volume over a wide range of buffer composition

AFFINITY CHROMATOGRAPHY



HIGH BINDING CAPACITY AT SHORT RESIDENCE TIME

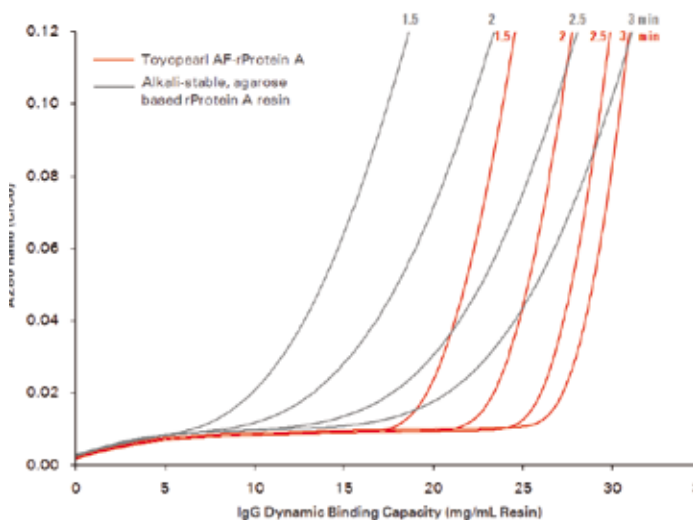
The particle size of 45 µm and the ligand density of Toyopearl AF-rProtein A-650F were optimized in order to reach high dynamic binding capacities (DBC) for immunoglobulins. Typical static IgG binding capacity is > 45 mg/ml resin and typical dynamic IgG binding capacity at 10 % breakthrough is > 30 mg/mL resin at 2 minutes residence time (1 mg/mL protein load). Fast mass transfer kinetics support high binding capacities even when applying high titer feedstocks at high flow rates. IgG breakthrough curves (Figure 2) at various linear velocities demonstrate the high IgG DBC at high velocities and the superior kinetic performance of Toyopearl AF-rProtein A-650F.

HIGH CIP AND SANITIZATION STABILITY

The structure of the recombinant ligand and its multipoint attachment to the base matrix enhances the stability of Toyopearl AF-rProtein A-650F in 0.1-0.5 M NaOH. The dynamic binding capacity remains high after repeated CIP cycles. After more than 150 CIP cycles with 0.1 M NaOH at 16 min contact time per cycle more than 90 % of initial dynamic binding capacity was retained (Figure 3). When performing cleaning-in-place with 0.5 M NaOH about 80 percent of IgG binding capacity remain after 50 cycles with 17 min contact time.

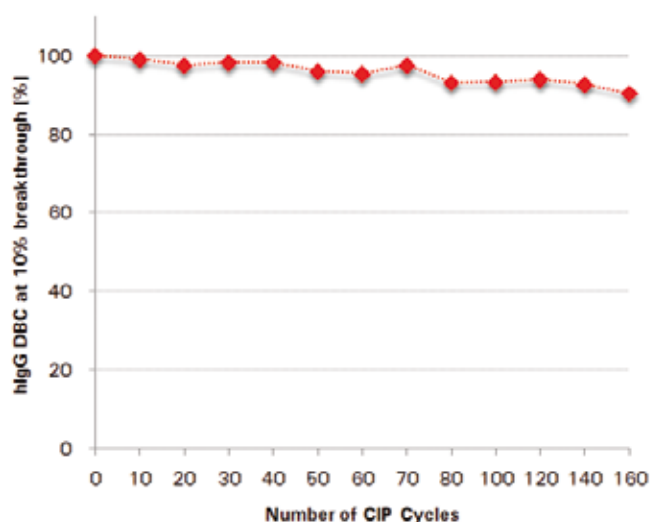
Toyopearl AF-rProtein A-650F is also stable in ethanol, 6 M urea, 6 M guanidinium chloride, and 1% phosphoric acid, respectively. Static binding capacity of the resin is not impaired when heated for 30 minutes to temperatures of up to 90°C. It can be stored at room temperature at production site. Recommended conditions for long term storage are a storage solution of 20 % ethanol and temperature of 4 - 8 °C.

FIGURE 2 DYNAMIC BINDING CAPACITY



Breakthrough curves for h-IgG loading (polyclonal, 10 mg/ml)
 Typical DBC at 10% breakthrough: 30,5 mg/mL @ 100 cm/hr (3 min residence time) - 24 mg/mL @ 200 cm/hr (1.5 min residence time)
 Column: 5 mm ID x 5 cm L;
 Mobile phase: 20 mM sodium phosphate buffer pH 7.2 containing 150 mM NaCl; Sample conc.: 10 mg/mL; Residence time: 1.5, 2.0, 2.5, 3.0 min

FIGURE 3 CLEANING-IN-PLACE STUDY WITH 0.1 M NaOH



Column: 5 mm ID x 5 cm L
 10 column volumes binding buffer pH 7.4
 5 column volumes elution buffer pH 3.0
 3 column volumes binding buffer containing 0.1 M NaOH, 16 min contact time
 3 column volumes binding buffer pH 7.4



AFFINITY CHROMATOGRAPHY

TOYOPEARL RESINS FOR AFFINITY LIGAND COUPLING

Toyopearl offers a spectrum of carefully selected affinity resins primed with activated or reactive groups which can be used to covalently attach almost any custom ligand. The structures of Toyopearl activated and reactive ligands are shown in Figure 4.

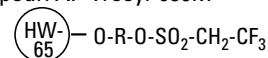
In general, Toyopearl AF-Tresyl-650M and Toyopearl AF-Formyl-650M resin are recommended for coupling proteins, while Toyopearl AF-Epoxy-650M resin is suited for coupling lower molecular weight ligands. Toyopearl AF-Amino-650M and Toyopearl AF-Carboxy-650M resins may be used for both.

Toyopearl affinity resins may be used in combinatorial chemistry or for solid phase synthesis of peptides and oligonucleotides because of their excellent stability in a variety of organic solvents and under extremes of pH.

FIGURE 4

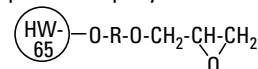
ACTIVATED TOYOPEARL AFFINITY RESINS

Toyopearl AF-Tresyl-650M



Ligand Density: 80 $\mu\text{mol/g}$ (dry)

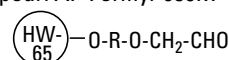
Toyopearl AF-Epoxy-650M



Ligand Density: 800 $\mu\text{mol/g}$ (dry)

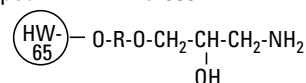
REACTIVE TOYOPEARL AFFINITY RESINS

Toyopearl AF-Formyl-650M



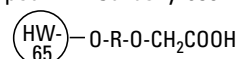
Ligand Density: 60 $\mu\text{eq/mL}$

Toyopearl AF-Amino-650M



Ligand Density: 100 $\mu\text{mol/mL}$

Toyopearl AF-Carboxy-650M



Ligand Density: 100 $\mu\text{eq/mL}$

TABLE II

REPRESENTATIVE COUPLING DENSITIES FOR ACTIVATED AND REACTIVE TOYOPEARL MEDIA

Toyoperl resin	AF-Tresyl-650M	AF-Formyl-650M	AF-Amino-650M	AF-Carboxy-650M
Protein coupled (mg/mL resin)				
Soybean trypsin inhibitor	16	3.5	5.8	15
Protein A	1.9	-	-	-
Concanavalin A	13	-	-	-
α 1-Antitrypsin	12.3	-	-	-
α -Chymotrypsin	12.5	-	-	-
Myoglobin	12.4	-	-	-
Ovalbumin	-	2.5	6.7	0.8
Bovine serum albumin	12.4	14	19.2	3.3
Human IgG	10.0	15	6.7	11.7
Cytochrome C	-	5.8	3.3	7.5
Lysozyme	60	20	5.8	17.5
Coupling agent	not required	NaCNBH ₃	NaCNBH ₃ or Cabodiimidamide	Carbodiimide
Optimal pH	7.0 - 9.0	6.9 - 9.0	4.5 - 6.0	4.5 - 6.0

AFFINITY CHROMATOGRAPHY



ACTIVATED RESINS – READY FOR DIRECT LIGAND ATTACHMENT

Toyopearl AF-Tresyl-650M activated resin is highly reactive toward amine and thiol groups. It is provided in dry form, ready for reaction in buffered solutions containing protein or other ligand. Coupling is accomplished in neutral to slightly alkaline (pH 7 - 8) solution (Figure 5).

Under such conditions, even proteins of limited stability may be successfully coupled. Coupling leads to the formation of a highly stable secondary amine or thio-ether linkage. The optimized tresyl-density (ca. 20 $\mu\text{mol/mL}$ hydrated resin) is sufficient to provide substantial protein binding while avoiding excessive multi-point attachment and consequent impairment of ligand affinity/activity. Representative data are presented in Table II.

Toyopearl AF-Epoxy-650M activated resin, also packaged in dry form, has a high density of epoxy- functionality (ca. 800 $\mu\text{mol/mL}$). Under appropriate reaction conditions, this may be used for immobilization of proteins or low molecular weight ligands. It is particularly useful when high densities of low molecular weight ligands must be attached (Figure 6). Glutathione and glycine have, for example, been coupled at densities greater than 100 $\mu\text{mol/mL}$ hydrated resin.

Toyopearl AF-Epoxy-650M resin is a highly versatile starting material for conversion to other chemically active functional groups required in special applications. This resin may be readily activated to hydrazide-bearing materials. This is particularly useful for immobilization of carbohydrates or glycoproteins. Using the reaction sequences described, special ligands may be introduced onto this dimensionally stable, macroporous support.

FIGURE 5 TOYOPEARL AF-TRESYL COUPLING PROCEDURE

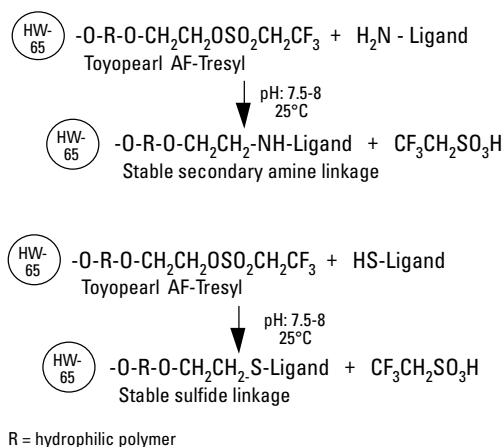


FIGURE 6 TOYOPEARL AF-EPOXY COUPLING PROCEDURE

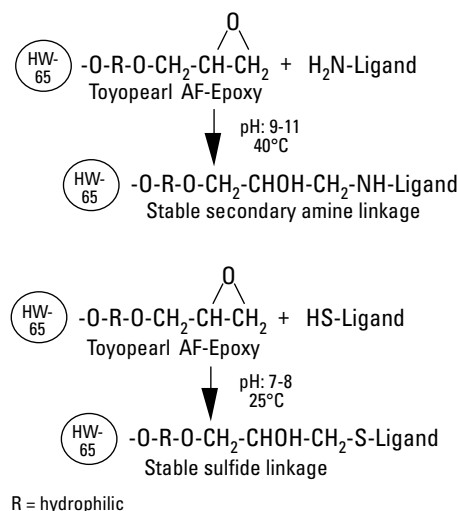


FIGURE 7 TOYOPEARL AF-FORMYL COUPLING PROCEDURE

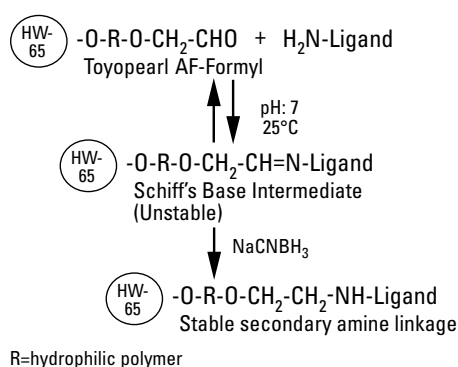
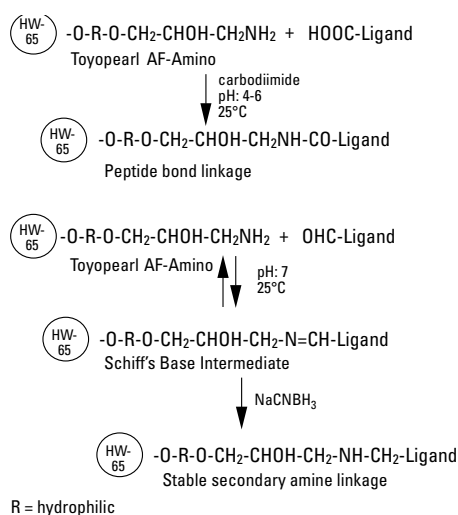


FIGURE 8 TOYOPEARL AF-AMINO COUPLING PROCEDURE





AFFINITY CHROMATOGRAPHY

REACTIVE RESINS - REQUIRE ACTIVATION FOR LIGAND ATTACHMENT

Ligands may be coupled to Toyopearl AF-Formyl-650M (aldehyde-bearing) resin under mild conditions exclusively using primary amines. The ligand is bound to the resin by a stable secondary amine linkage (Figure 7). Representative coupling capacities are shown in Table II.

A wide variety of industrial enzymes have been immobilized on aldehyde-bearing supports. Typically, these supports have been synthesized by industrial users by partial oxidation of polysaccharide supports (e.g. cellulose and agarose) or partial hydrolysis of polyacetals. In contrast, Toyopearl AF-Formyl-650M resin is a ready-to-use aldehyde support formulated from a dimensionally stable, macroporous matrix. Consistent aldehyde content and physical properties are assured from batch to batch.

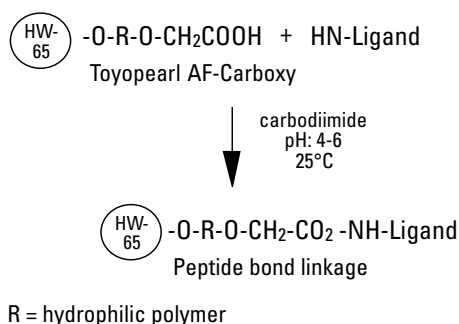
Toyopearl AF-Amino-650M resin may be used to couple ligands using their carboxyl groups (peptide bond formation) or aldehyde groups (reductive amination) as shown in Figure 8. Aldehyde groups may be present in a carbohydrate or glycoprotein ligand or may be introduced into the ligand by mild, periodate oxidation.

The optimized functional group density of Toyopearl AF-Amino-650M (100 $\mu\text{mol/mL}$) is ideal for coupling of either proteins or low molecular weight ligands. For example, lactose was coupled by reductive alkylation to yield a ligand density of ca. 30 $\mu\text{mol/mL}$ resin. Coupling densities for various proteins are given in Table II.

Toyopearl AF-Carboxy-650M resin provides another useful and milder approach for coupling to amino groups of proteins or low molecular weight ligands. The carbodiimide mediated coupling reaction produces an amide bond between ligand and support (Figure 9). Representative coupling densities are given in Table II.

FIGURE 9

TOYOPEARL AF-CARBOXY COUPLING PROCEDURE



TOYOPEARL RESINS WITH GROUP SPECIFIC LIGANDS

The structures of Toyopearl group specific ligands are shown in Figure 10.

Toyopearl AF-Chelate-650M

This resin is derivatized with iminodiacetic acid (IDA) at a concentration of ca. 20 $\mu\text{mol/mL}$. In typical applications, selected metal ions, most often Ca^{2+} , Ni^{2+} , Zn^{2+} , Co^{2+} and Cu^{2+} are bound to the support by stable chelation. The resultant metal ion-bearing resin binds to histidine and free cysteine containing sequences of a peptide or protein. Immobilized metal ion affinity chromatography (IMAC) has been used for purification of recombinant human growth factor, tissue plasminogen activator, glycoporphins, and whole cells.

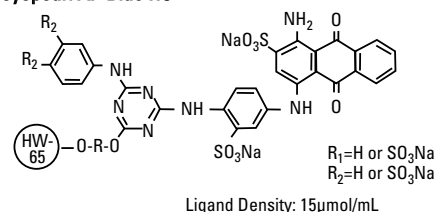
Toyopearl AF-Blue HC-650M

Functionalized with Cibachron Blue F3G-A, Toyopearl AF-Blue HC-650M resin has excellent capacity for proteins, particularly albumin (Figure 10). In addition, this high capacity resin has improved caustic stability, reduced dye ligand leakage, and superior pressure-flow characteristics relative to more traditional agarose materials (Figure 11).

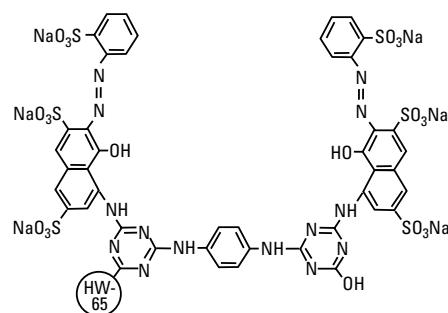
FIGURE 10

GROUP-SPECIFIC TOYOPEARL AFFINITY RESIN

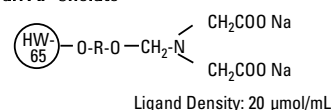
Toyopearl AF-Blue HC



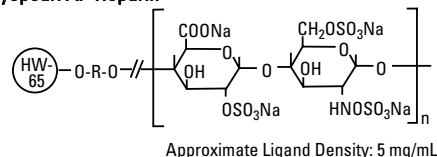
Toyopearl AF-Red



Toyopearl AF-Chelate



Toyopearl AF-Heparin



AFFINITY CHROMATOGRAPHY



Toyopearl AF-Red-650ML

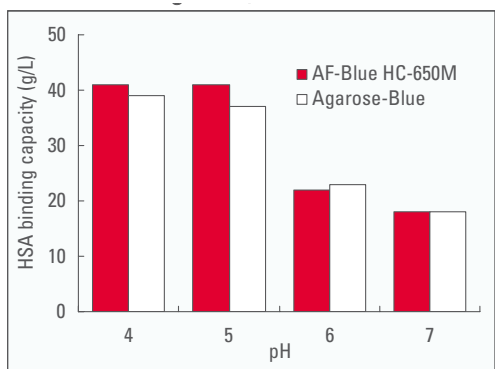
Toyoscreen and Toyopearl AF-Red-650ML resins are functionalized with Procion Red HE-3B, (also known as Reactive Red 120). This resin is useful for the purification of nucleotide dependent enzymes, lipoproteins, plasminogen, peptides, hormones and cytotoxins.

These two dye-ligand resins are useful in binding/purification of nucleotide-dependent enzymes, albumin, cell growth factors, interferons, transferases, cyclases, and polymerases. Typical binding capacities are shown in Table III.

TABLE III
REPRESENTATIVE BINDING CAPACITIES FOR TOYOPEARL DYE-LIGAND AFFINITY MEDIA

Protein (mg/mL resin)	AF-Blue HC-650M	AF-Red-650ML
Hexokinase	3	-
Bovine serum albumin	16	-
Human serum albumin	18+-2.5	3.5+-1
Lactate dehydrogenase	27	11

FIGURE 11
COMPARISON OF HUMAN SERUM ALBUMIN BINDING CAPACITIES AT VARIOUS pH'S OF AF-BLUE HC-650M AND AGAROSE (BLUE FUNCTIONALIZED AGAROSE)



Conditions

- A 1.0 mL of adsorbent was washed with 10 mL of equilibration buffer (pH 4.0 and 5.0; 0.1 mol/L sodium acetate buffer, pH 6.0 and 7.0; 0.1 mol/L sodium phosphate buffer).
- A 5.0 mL of 1 % solution of human albumin dissolved in each equilibration buffer was charged onto the column.
- After 10 min, unbound albumin was eluted and the column was washed with 10 mL of each equilibration buffer.
- Adsorbed human albumin was eluted with 0.1 mol/L sodium phosphate buffer at pH 7.0 containing 2.0mol/L sodium chloride (desorption buffer) and 10 mL fractions were collected.
- Human albumin content was measured spectrophotometrically by using E 0.1% at 280 nm = 0.55.

Toyopearl AF-Heparin HC-650M

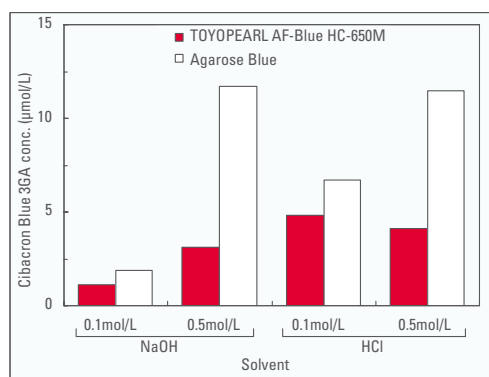
Heparin is a linear and highly sulfated glycosaminoglycan which has anti-coagulant properties. Due to its polyanionic nature, heparin interacts with a wide range of biomolecules including plasma components, lipoprotein lipase, collagenase, and DNA polymerase.

Immobilized heparin is widely used as an adsorbent in affinity chromatography for the purification of biological substances. Toyopearl AF-Heparin HC-650M resin is a high capacity, affinity adsorbent with excellent chemical stability.

TOYOSCREEN PREPACKED COLUMNS FOR PROCESS DEVELOPMENT

ToyoScreen columns are available in 1 mL and 5 mL resin volumes of the following Toyopearl AFC products: Toyopearl AF-Chelate 650M, Toyopearl AF-Heparin HC-650M, Toyopearl AF-Blue HC-650M and Toyopearl AF-Red-650M. They provide a convenient way to perform early resin screening for both target retention and recovery. Multiple columns can be connected in series for additional separation. Please see the ordering information at the end of this section or contact us to request more information on our ToyoScreen offerings.

FIGURE 12
COMPARATIVE DYE LEAKAGE STUDY OF AF-BLUE HC-650M AND COMPETITOR BLUE @ 25°C (AFTER 24 HOURS)



Conditions: 200 mg of each material was suspended in 4 mL of solvent and incubated at 25°C and shaking for 24 h. The absorption at 620 nm of the supernatants were measured after appropriate adjustment to neutrality with known volume of acid or base. Dye concentrations were estimated assuming a molar extinction coefficient of 12,750 (L/M cm).



AFFINITY CHROMATOGRAPHY

ORDERING INFORMATION

TOYOSCREEN PROCESS DEVELOPMENT COLUMNS FOR AFC

PART #	PRODUCT DESCRIPTION	PACKAGE
22809	ToyoScreen AF-rProtein A-650F NEW	1 mL x 5 each
22810	ToyoScreen AF-rProtein A-650F NEW	5 mL x 1 each
22811	ToyoScreen AF-rProtein A-650F NEW	5 mL x 5 each
21384	ToyoScreen AF-Chelate-650M	1 mL x 6 each
21385	ToyoScreen AF-Chelate-650M	5 mL x 6 each
21386	ToyoScreen AF-Blue HC-650M	1 mL x 6 each
21387	ToyoScreen AF-Blue HC-650M	5 mL x 6 each
21388	ToyoScreen AF-Red-650ML	1 mL x 6 each
21389	ToyoScreen AF-Red-650ML	5 mL x 6 each
21390	ToyoScreen AF-Heparin HC-650M	1 mL x 6 each
21391	ToyoScreen AF-Heparin HC-650M	5 mL x 6 each

TOYOSCREEN COLUMN ACCESSORIES

PART #	PRODUCT DESCRIPTION
21400	ToyoScreen Column Holder

TOYOPEARL LABPAK

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	PARTICLE SIZE (µm)
43400	AFFIPAK ACT (AF-Epoxy-, AF-Tresyl-650M)	2 x 5 g*	65
43410	AFFIPAK (AF-Amino-, AF-Carboxy-, AF-Formyl-650M)	3 x 10 mL	65

TSKgel RESIN

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	PARTICLE SIZE (µm)
16208	Tresyl-5PW (10)	2 g	10

TOYOPEARL AFFINITY CHROMATOGRAPHY RESIN

GROUP SPECIFIC RESINS

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	TYPICAL CAPACITY
22803	Toyopearl AF-rProtein A-650F NEW	10	40 mg/mL (IgG)
22804		25	
22805		100	
22806		1,000	
22807		5,000	
22808		50,000	

PROTEIN A IMMUNOASSAYS & STANDARDS

PART #	PRODUCT DESCRIPTION
22815	Protein A-R28 ELISA Kit NEW
22836	Protein A-R28 STD 0.5 mL (10 mg/L) NEW

AFFINITY CHROMATOGRAPHY



ORDERING INFORMATION

GROUP SPECIFIC RESINS

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	TYPICAL LIGAND DENSITY	TYPICAL CAPACITY
19688	Toyopearl AF-Blue-HC-650M	25	15 µmol/mL	minimum 18 mg/mL
19689		100		
19690		1,000		
19691		5,000		
08651	Toyopearl AF-Red-650ML	25	5 µmol/mL	2.5 - 4.5 mg/mL (HSA)
19801		100		
42102		1,000		
14475	Toyopearl AF-Chelate-650M	25	25-45 µeq/mL	-
19800		100		
14907		1,000		
14908		5,000		
20030	Toyopearl AF-Heparin HC-650M	10	-	5 mg/mL (AT III)
20031		100		
20032		1000		
20033		5000		

TOYOPEARL AFFINITY CHROMATOGRAPHY RESIN

REACTIVE RESINS

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	TYPICAL LIGAND DENSITY	TYPICAL CAPACITY
43411	Toyopearl AF-Amino-650M	10	70-130 µeq/mL	-
08002		25		
08039		100		
18074		1,000		
18316		5,000		
43412	Toyopearl AF-Carboxy-650M	10	80-120 µeq/mL	-
08006		25		
08041		100		
18827		1,000		
18828		5,000		
43413	Toyopearl AF-Formyl-650M	10	40-70 µeq/mL	-
08004		25		
08040		100		
17396		1,000		
17397		5,000		

ACTIVATED RESINS

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE	TYPICAL LIGAND DENSITY	TYPICAL CAPACITY
43402	Toyopearl AF-Epoxy-650M	5 g*	600 - 1000 µeq/g	-
08000		10 g*		
08038		100 g*		
18315		1,000 g*		
14471	Toyopearl AF-Tresyl-650M	5 g*	80 µmol/mL	-
14472		100 g*		
14906		1,000 g*		

Conditions: All Toyopearl affinity resins are provided at a particle size of 65 µm. This particle size is ideal for both small and large scale separations.

*1 g yields approximately 3.5 mL of hydrated resin.